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DPG Document No. DPG HB 80-804

HANDBOOK

FOR EVALUATING ECOLOGICAL EFFECTS OF POLLUTION AT DARCOM INSTALLATIONS

VOLUME 4

TERRESTRIAL SURVEYS

DECEMBER 1979

U.S. ARMY DUGWAY PROVING GROUND
Dugway, Utah 84022

Supersedes AD-A089860

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This handbook provides the DARCOM commander with a tool whereby he can respond quickly to a potential or actual pollution incident with a decisive program to evaluate the ecological effects of the pollution. To implement the procedures as set forth in the handbook, the commander will enlist the help of an environmental team composed of DARCOM scientists (or other suitable personnel) and individuals with limited ecological training (paraecologists) who will do much of the manual labor. With a given volume, the team can perform the required functions. (con't)

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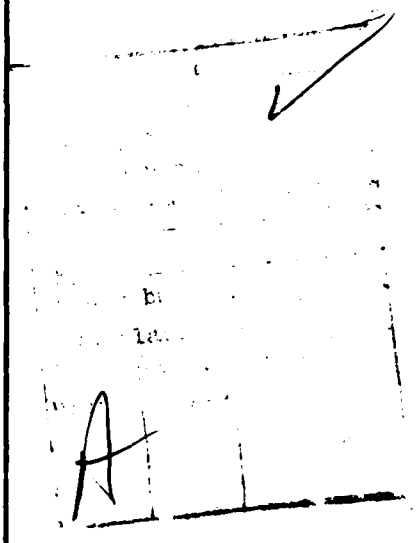
Item 20 (con't)

The handbook covers the following areas in seven volumes of which this is Volume 4: (1) basic questions that need answering, (2) conducting the preliminary investigation of the problem, (3) determining the specific effects of a pollutant (the first three volumes are essentially library efforts), (4) terrestrial sampling, (5) aquatic sampling, (6) unexpected declines in animal populations and (7) handling data.



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CONCEPT

Each chapter in Volume 4 deals with a biological indicator. The biological indicators chosen were selected because they possess most of the following criteria:

(1) Sensitive to the pollutants most likely encountered (chemical or biological) or insensitive to the pollutants but bioaccumulate them above background levels.

(2) Present during a pollution episode (year-round residents)

(3) Exposed during a pollution episode (active in the habitat receiving the pollutant)

(4) Characterized by an essentially stable population from year to year unless the environment is stressed by a pollutant

(5) Short-lived with a high biotic potential (rapid reproduction) and a high immigration potential. (Once the stress is removed from the environment, these indicators replenish the stressed area rapidly)

(6) Long-lived with a low biotic potential and a low immigration potential. (Once the stress is removed from the environment, these indicators replenish the stressed area slowly)

(7) Characterized by a home range that is likely to be as large as a suspected polluted area (to provide an overview), or smaller than a suspected polluted area (to study selected sites within a polluted area)

(8) Abundant enough for adequate sample sizes, and

(9) Easily and accurately sampled by paraecologists using inexpensive equipment

Pollution ecology surveys (PES) can be required on past, present or proposed polluting activities.

Even though the past polluting activity may be inactive, there may be residual effects because of the persistence of compounds in the environment (e.g., chlorinated hydrocarbons). It also may be important to determine whether the activity ever caused environmental stress during operation. For these reasons, PES on past pollution episodes require biological indicators that preserve a record of the past (i.e., long lifetime and low biotic/immigration potential). Past vegetative condition (as revealed through historical photographs), tree rings and turtle populations are good examples of such indicators.

Activities that are polluting now could have been activated a long time ago or quite recently. In the event of an activity that has been operating for a long period, the impact could be on-going, in which case it would be important to know whether the environment is responding positively to pollution abatement measures. Obviously a biological indicator chosen under these circumstances should have the potential for rapid recovery (short lifetime and high biotic/immigration potential). In the event of a recently initiated activity, chronic effects would not be identified immediately, necessitating a long-term study. A biological indicator chosen for this type of study should be expected to maintain a population in the area indefinitely (year-round resident). In general, foliage condition, earthworms, bees, rodents and insectivores possess these qualities. These biological indicators also can be used to assess the extent of ecological damage from accidental spills of polluting substances (refer to Volume 6).

Biological indicators chosen for proposed activities must possess the same characteristics as those for presently polluting activities. Consequently, the biological indicators can be the same.

Pollution ecology surveys require experimental and reference areas. An experimental area is the area where the polluting activity is suspected of causing environmental damage. A reference area is one not affected by the polluting activity, and one in which all relevant variables (climate, topography, substrate and biota) are as close as possible to those in the experimental area.

For past polluting activities and proposed activities, the experimental and reference areas are often the same. The area is studied before an activity begins, to establish a baseline;[1] and concurrently during the operation, to detect changes in the baseline that indicate environmental stress. Usually, time is considered an irrelevant variable.[2]

---

[1]The baseline can include species structure (numbers and kinds of species present) and levels of pollutants in the species sampled.

[2]This may not be so, for example, if relevant variables in the study area are known to undergo cycles which may in turn affect the biological indicators.

In cases involving presently polluting activities, the experimental area cannot double as the reference area unless some of the techniques for studying past polluting activities can be applied. When they cannot, a reference area is chosen that most closely resembles the habitat of the experimental area but is isolated from the reference area. There is an inherent risk in this approach because there may be subtle microhabitat differences that can have a profound effect on the biological indicators. Furthermore, areas regarded as appropriate references could have a habitat similar to that of the experimental area because they are exposed to similar substances.

These potential pitfalls may be overcome by carefully tailoring the selection of the reference area. The first step in the selection process is to establish a baseline of the ecosystems in the vicinity of the activity so that a number of candidate sites can be selected for further study and proper biological indicators can be chosen. The literature survey outlined in Volumes 2 and 3 will determine whether the necessary baseline information is available. If not, we recommend using the procedures outlined in the Study of Ecological Classification and Inventory Manual[1], which utilizes field observations to establish a baseline. Once the baseline has been established this volume will be used to conduct the PES.

It is highly preferable that a study on a proposed activity be initiated a year or more before, rather than after, that activity commences, because a reference area at the same site as the experimental area is more desirable.

[1]U.S. Department of the Navy, Naval Facilities Engineering Command, Alexandria, VA 22332. Study of Ecological Classification and Inventory Manual, M.M. Goodwin, Oct 1977.

USE OF THIS VOLUME

This volume consists of separate chapters which provide the paraecologist with detailed instructions for collecting samples in the field. Correct application of the methodology described in each chapter is essential for assuring the scientific validity of the data. Although detailed instructions are provided, two considerations make it imperative that the environmental scientists closely monitor the paraecologist during the initial phases of data collection: (1) certain determinations such as location of sampling points, time and frequency of sampling, etc. must be made by the environmental scientist for each situation and as such, cannot be detailed in the handbook, and (2) an inexperienced paraecologist is prone to make errors during the first few sampling attempts.

Close supervision of the paraecologist at the beginning of the field survey can be replaced by intermittent supervision when the environmental scientist is satisfied that the paraecologist can independently collect scientifically valid data without making mistakes (e.g., cross contamination, inadequate record keeping and mislabeled samples).

Chapter 1 describes some techniques for obtaining the data necessary for selecting appropriate reference areas when the experimental and reference areas cannot be the same.

Each of the remaining chapters deals with one or more biological indicators. The choice of which indicator(s) to use will be based upon the discussion in the prior section and a thorough consideration of the situation under study, including the use of Volume 3 to determine if the biological indicator responds to the pollutant in a desired way (e.g. the pollutant is bioaccumulated).

The biological indicators covered in this volume may not prove to be the best in every case. Where they are not adequate and new biological indicators are identified and techniques for their sampling written up, request the information be sent to:

Commander
USA Dugway Proving Ground
ATTN: STEDP-MT-L
Dugway, UT 84022

for possible inclusion in the next revision.

Each chapter will contain two sections. The first discusses those circumstances under which the specific biological indicator is appropriate. The second covers the sampling methodology for the biological indicator(s). As appropriate, this section will discuss sampling methods, identification of species, permit requirements, record keeping, disposition of specimens collected, and an equipment checklist.

FOREWORD

This volume was revised by Dr. Carlos F. A. Pinkham and David A. Gauthier.

CHAPTER 1

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CHAPTER 2

Prepared by George T. Crane and E-5 J. Douglas Sanders, U.S. Army Dugway Proving Ground, Dugway, UT 84022

The following people and organizations have contributed information used in Chapter 2:

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William Cunliffe, Office of Adjutant General, Department of Army, Washington, DC 20314

Thomas Early, EROS Data Center, Sioux Falls, SD 57198

Richard E. Spurr, National Archives, Washington, DC 20408

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Dr. Norman E. Gary, Department of Entomology, College of Agriculture and Environmental Sciences, University of California, Davis, CA 95616

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Dr. Adair Stoner, Agricultural Research Service, U.S. Department of Agriculture, Laramie, WY 82071

CHAPTER 8

Prepared by Dr. Harold E. Stark, Dr. Carlos F. A. Pinkham and Mr. David A. Gauthier, U.S. Army Dugway Proving Ground, Dugway, UT 84022

NOTE

The pronoun "he" is used in this volume as an impersonal pronoun which incompasses he and she and has no intent of personal reference or connection.

CHAPTER 1 - SELECTION OF EXPERIMENTAL AND REFERENCE STUDY AREAS

CONTENTS

a. APPLICATION

The area affected by the polluting activity may be known or unknown. If it is known, the experimental area is situated in the affected area. If it is unknown, calculate isopleths of average concentration resulting from emissions for given hypothetical source strengths using the technique identified on page 17,Volume 7. Generally the area with the highest concentration will be the experimental area.

Selection of candidate reference areas from among the ecosystems around the activity can be determined by using the appropriate criteria outlined in the "Data Point Requirements for Terrestrial Communities"[1]. Those ecosystems that have the maximum number of appropriate data points in common will form the candidate reference areas. The procedures outlined in this chapter and Chapter 2 should be used to select the best reference area from among the candidate areas.

Detailed information about the reference and experimental areas is necessary for interpreting the data generated by the studies conducted using the following chapters.

[1]U.S. Department of the Navy, Naval Facilities Engineering Command, Alexandria, VA 22332. Study of Ecological Classification and Inventory Manual, M.M. Goodwin, Oct 1977.

This chapter uses vegetative communities to characterize the ecosystem. Similar vegetative communities usually have similar climate, topography, substrate and animal communities.

b. SAMPLING METHODOLOGY

(1) General[1]

Square or rectangular areas called quadrats will be used to characterize the plant community. Quadrat size and plant size are intimately related thus the larger the plant size the larger the quadrat required to describe the species. Table 1-1 shows the size of the quadrat based upon the nature of the vegetation being described[2]. A portable metal frame of the proper size is useful for the smaller quadrats. A cloth tape measure and a large right angle will be necessary to stake out the larger quadrats. Pound corner stakes in the ground and run twine between the adjacent pairs to delineate the boundary of the quadrat.

Table 1-1. Quadrat Size as Determined by Type of Vegetation

Type of Vegetation	Quadrat Size (m^2)
Soil layer, cryptogram - dominated layer	0.01 to 0.1
Herbaceous layers	1.0 to 2.0
Rank herbs or low shrubs	4.0
Tall shrubs or low trees	16.0
Superior layers of forest	100.0

The quadrats must be located in such a manner to avoid bias in quadrat site selection. The method of Cain and Castro[3] will be used

[1]Shimwell, D. W., The Description and Classification of Vegetation, University of Washington Press, Seattle, WA, 1972.

[2]Cain, S. A., "Concerning Certain Phytosociological Concepts", Ecological Monograph, 2:475-508, 1932.

[3]Cain, S. A. and Castro, G. M. de O., Manual of Vegetation Analysis, Harper, NY, 1959.

to assure random selection of quadrat sites. Select a point in the center of the general area to be surveyed and lay down two lines, one going east to west, the other north to south (Figure 1-1). Thirty-six consecutive quadrats are laid off on each of the two lines. Starting at the western end of the east-west line, number the quadrats as shown in Figure 1-1. The same procedure is used for the north-south line, starting at the south end of the line and working north. Each group of 10 quadrats are assigned clubs, spades, hearts or diamonds as shown in Figure 1-1.

A stack of shuffled playing cards (face cards removed) is used to randomly select 25[1,2] of the 80 quadrats. The fifth card dealt is used to locate a coordinate on the east-west line. This procedure is repeated for locating a coordinate on the north-south line. The cards are reshuffled and the process is repeated again until 12 positions for quadrates are selected along the east-west line and 13 along the north-south line. A random numbers table can be used in lieu of the deck of cards if desired (refer to page 11, Volume 7).

Several parameters which should be examined when defining plant communities are: (1) identification of species present, (2) density, (3) cover, and (4) frequency.

Identification of Species Present

All the species rooted[3] within a quadrat will be identified (see Appendix A). The paraecologist will be trained in the identification of the common species. Those which cannot be identified will be collected, pressed and shipped to a plant taxonomist for proper identification. (Refer to Appendix B for permit requirements, Chapter 5 for collecting and shipping techniques, and Appendix A for species identification.)

Density

Density refers to the number of individuals of a given species found per unit area. It is calculated for each set of 25 quadrats by

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[1]Raunkiaer, C., "Recherches Statistiques sur les Formations Vegetales," K. Danske Videnske Selsk. Biol. Meddel., 1:1-47,1918.

[2]Raunkiaer, C., The Life Forms of Plants and Statistical Plant Geography, Oxford Press, 1934.

[3]Greig-Smith, P., Quantitative Plant Ecology, Butterworth, London, 2nd Ed, 1964.

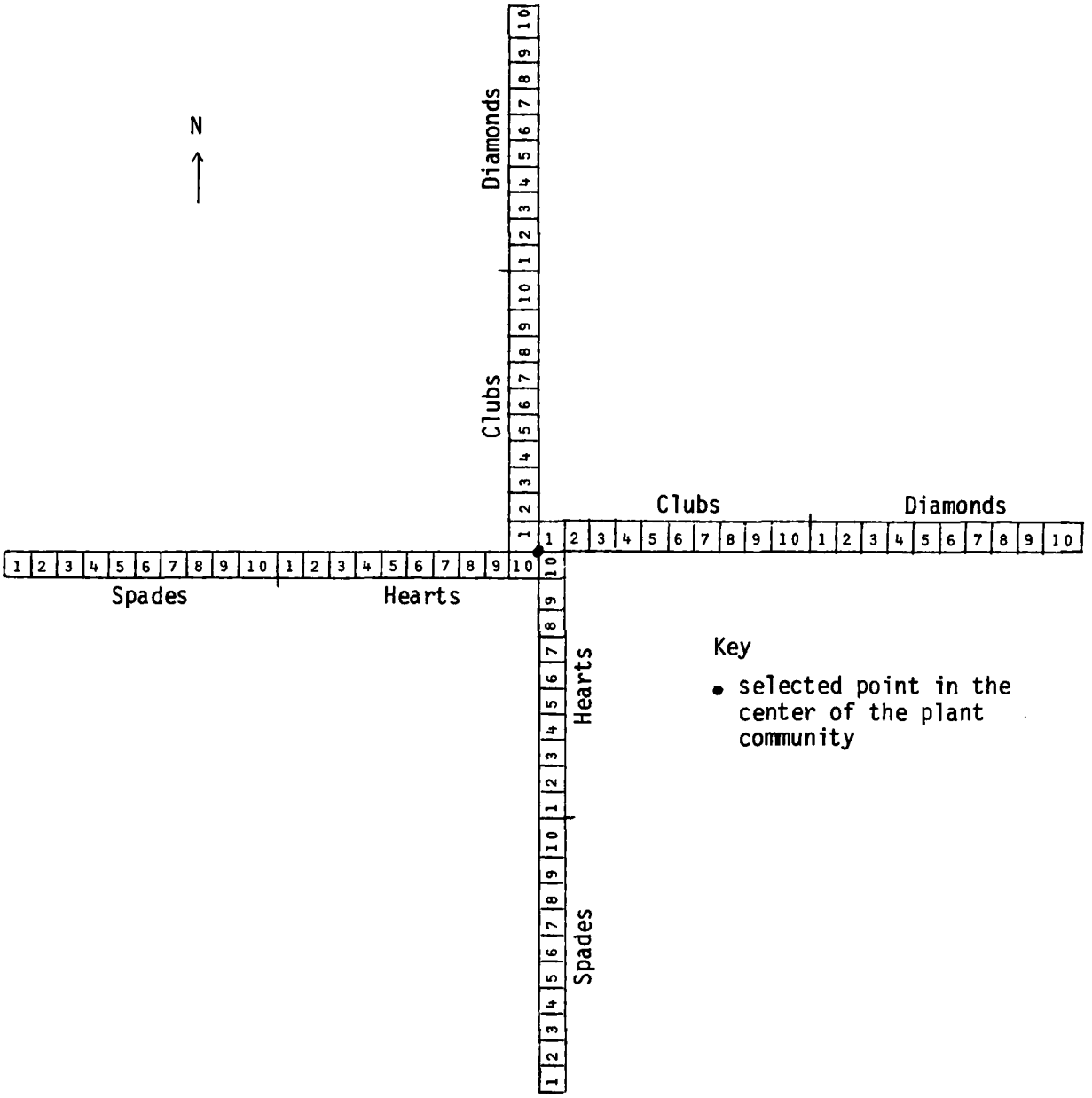


Figure 1-1. Configuration for Randomly Selecting Quadrat Sites

totaling the numbers of each plant species counted in the 25 quadrats and dividing by the total area of the quadrats. Density varies greatly from species to species mainly because of reproductive type, seed dispersal type and population age.

Cover

Cover is the area of the quadrat shaded or overhung by the leaves and stems of a given species. Percent cover values are estimated visually according to the four classes of cover[1] given in Table 1-2.

Table 1-2. Classes Associated with Percent Cover of Plants

Percent Cover	Class
0 - 10%	1
11 - 30%	2
31 - 50%	3
51 - 100%	4

Frequency

The frequency of a given species refers to the chance of encountering it in a single quadrat. A species has a frequency of 100 percent if it is present in all quadrats surveyed, 80 percent if it is observed in 20 of the 25 quadrats and so on.

(2) Record Keeping Refer to Appendix C.

The form for recording data from each quadrat is given in Figure 1-2. The "Study Title" will be determined by the team leader. The date is written as the day, month and year, using the three-letter abbreviation for the month. For example: 14 Apr 78. "Team Members Present" are those conducting the survey on the quadrat. "Plant Community Location" is the location given for the quadrat as in the "Location" blank on the standard label in Appendix C. "Quadrat Number" is the number of the quadrat as follows: Quadrats on the east-west line are identified with "E", those on the north-south line, with "N". Spades, hearts, clubs and diamonds are abbreviated by "S", "H", "C" and "D" respectively. Thus, the third quadrat from the eastern end of the east-west line is identified by: "ED8".

[1]Greig-Smith, P., Quantitative Plant Ecology, Butterworth, London, 2nd Ed, 1964.

Study Title _____

Date _____ Team Members Present _____

Plant Community Location _____ Quadrat Number _____

Species	Number of Individuals	Percent Cover Class	Species	Number of Individuals	Percent Cover Class

Figure 1-2. Form Used to Record Data Obtained From Each Quadrat

The form for summarizing data from all 25 quadrats in a community is given in Figure 1-3. The headlines are the same as in Figure 1-2 except for the "Recorder", the person filling out the form. The remainder of the entries are self-evident.

(3) Analysis of Data

The data can be analyzed using the analysis described in Shimwell[1]. The programs for the appropriate statistical tests are found in Volume 7 of this handbook.

(4) Equipment Checklist Refer to Appendix F.

Portable metal frame
Cloth tape measure
Right angle
Quadrat corner stakes
Twine
Directional compass
Deck of cards
Quadrat data forms (Figures 1-2 and 1-3)
Plant collecting equipment (identified in Chapter 5)

Shimwell, D. W., The Description and Classification of Vegetation, University of Washington Press, 1972.

Study Title _____

Date _____ Recorder _____

Plant Community Location _____

Species _____

Quadrat	Number of Individuals	Percent Cover Class
(1)		
(2)		
(3)		
(4)		
(5)		
(6)		
(7)		
(8)		
(9)		
(10)		
(11)		
(12)		
(13)		
(14)		
(15)		
(16)		
(17)		
(18)		
(19)		
(20)		
(21)		
(22)		
(23)		
(24)		
(25)		

Area Per Quadrat _____

Total Area _____

Total Number of Individuals _____

Density _____

Sum of Percent Cover Classes _____

Mean Percent Cover Class _____

Number of Quadrats Containing This Species _____

Frequency of Occurance _____

Figure 1-3. Form Summarizing Data From Each Set of 25 Quadrats

CHAPTER 2 - HISTORICAL AERIAL PHOTOGRAPHS: INDICATORS OF PAST AIRBORNE AND SOIL POLLUTION

CONTENTS

a. APPLICATION

Historical photographs can be utilized: (1) to determine man-made changes or successional stages in plant communities, (2) to observe the effects of pollution and (3) to monitor recovery from past pollution.

b. SAMPLING METHODOLOGY

(1) Sources

Aerial photographs may be obtained from the Department of Army, the National Archives and Records Service, and the U.S. Geological Survey.

The Department of Army:

The Adjutant General Office, Department of Army, Room GA 076, Forrestall Bldg., Washington, DC 20314, controls the holdings of aerial photographs of Army installations from 1950 to present. For specific requests call 202 693-0970.

The National Archives and Records Service:

The National Archives and Records Service maintains a comprehensive repository of military photographs of the United States from World War II to 1950; these can be obtained from the following address:

National Archives and Records Service
Military Archives Division
Penn Ave. at 8th St. N.W.
Washington, DC 20408
Phone: (202) 523-3340

The U.S. Geological Survey:

The National Cartographic Information Center of the U.S. Geological Survey (Table 2-1) maintains records of aerial photographic coverage of the United States and its territories. These records document surveys made by federal and state agencies, and commercial companies. In addition, four mapping centers hold negatives for maps for their respective areas (Table 2-1 and Figure 2-1).

The Earth Resources Observation Services (EROS) Data Center of the U.S. Geological Survey (Table 2-2) provides access to aerial photography and imagery acquired by the National Aeronautics and Space Administration (NASA) from research aircraft and from Skylab, Apollo and Gemini Spacecraft. Over 5 million images and photographs of the earth's surfaces are in computerized data storage.

EROS also has Applications Assistance Facilities (Table 2-2) which maintain copies of data stored at the Center and provide computer terminal inquiry and order capability to the central computer complex at the EROS Data Center. Scientific personnel are available for assistance in applying microfilm copies of data to a variety of resource and environmental problems and for assistance in ordering data from the Data Center.

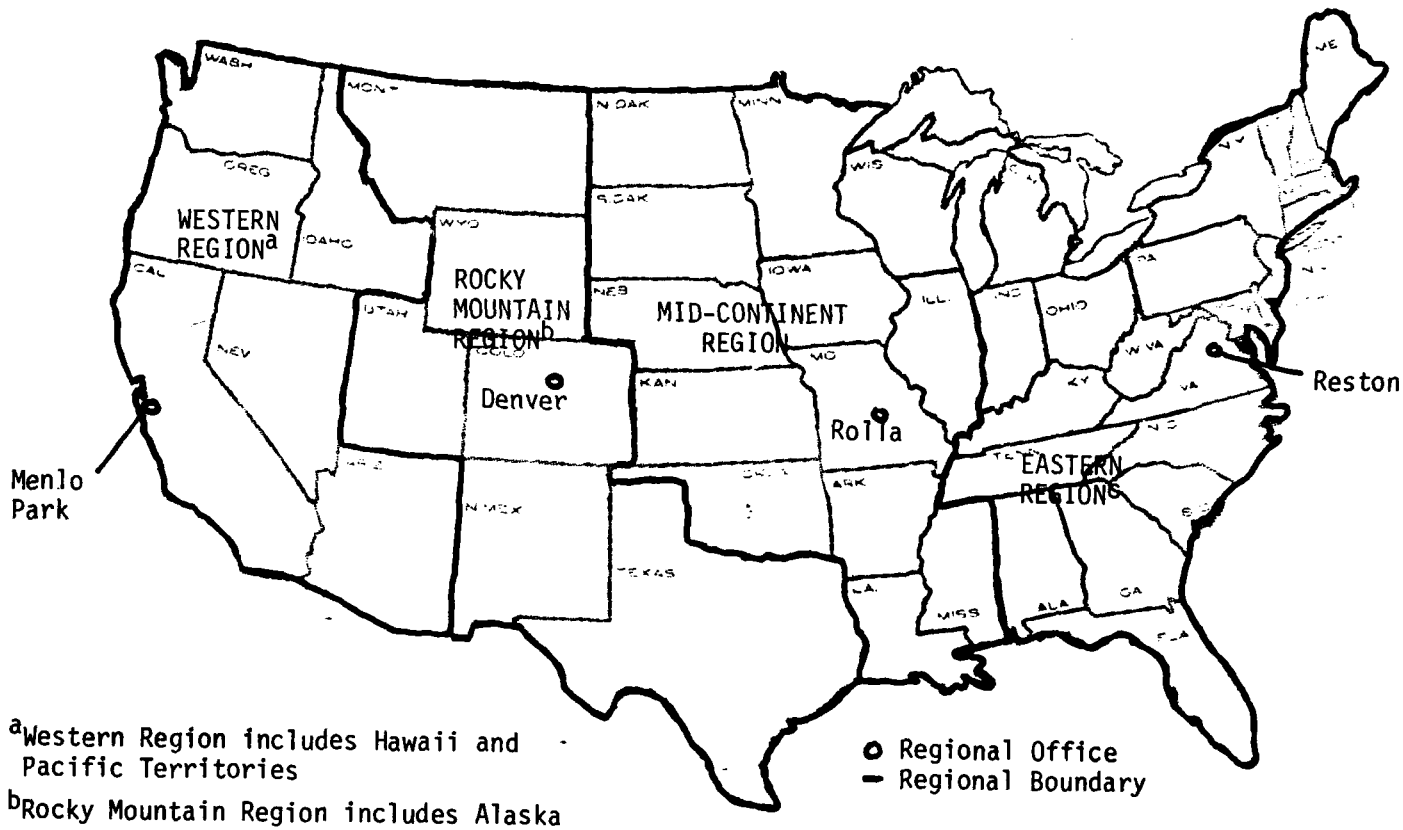
In addition, EROS Data Reference Files (Table 2-2) have been established throughout the United States to maintain microfilm copies of the data available from the Data Center and to provide guides to assist the user in reviewing and ordering data. This allows the user to view microfilm copies of the data before placing an order. Applications assistance is not provided at EROS Data Reference Files.

(2) Ordering Information

Many types of aerial photographs are available. The important considerations in ordering historical aerial photographs are (a) attitude, (b) scale, (c) chromatism (d) area (e) intended use. (f) time frame and (g) index numbers.

Table 2-1. U.S. Geological Survey Regional Mapping Centers

Activity	Address	Telephone
National Cartographic Information Center	National Cartographic Information Center U.S. Geological Survey 507 National Center Reston, VA 22092	703 860-6045
Mapping Centers	Eastern Mapping Center U.S. Geological Survey 536 National Center Reston, VA 22092	703 860-6336
	Mid-Continent Mapping Center U.S. Geological Survey Box 113 (or 900 Pine St.) Rolla, MO 65401	314 264-3680 EXT 107
	Rocky Mountain Mapping Center U.S. Geological Survey Federal Center, Bldg. 25 Denver, CO 80225	303 234-4553
	Western Mapping Center U.S. Geological Survey 345 Middlefield Rd. Menlo Park, CA 94025	415 323-8111 EXT 2427



[a]Western Region includes Hawaii and Pacific Territories

[b]Rocky Mountain Region includes Alaska

[c]Eastern Region includes Puerto Rico and Virgin Islands

Figure 2-1. Regional organization of the U.S. Geological Survey Regional Mapping Centers

Table 2-2. Earth Resources Observation Services Data Centers and Facilities

Activity	Address	Telephone
Data Center	User Services Unit EROS Data Center Sioux Falls, SD 57198	605 594-6511 Ext 151
Applications Assistance Facilities	EROS Applications Assistance Facility U.S. Geological Survey 1925 Newton Square East Reston, VA 22090	703 860-7868
	EROS Applications Assistance Facility U.S. Geological Survey Rm. 8-210, Bldg. 1100 National Space Technology Laboratories Bay St. Louis, MI 39520	601 688-3472
	EROS Applications Assistance Facility U.S. Geological Survey Rm. 2404B, Bldg. 25 Federal Center Denver, CO 80225	303 234-4879
	EROS Applications Assistance Facility EROS Data Center U.S. Geological Survey Sioux Falls, SD 57189	605 594-6511
	EROS Applications Assistance Facility U.S. Geological Survey Rm. 5017, Federal Bldg. 230 North 1st Ave. Phoenix, AZ 85025	602 261-3188

(continued)

Table 2-2. Earth Resources Observation Services Data Centers and Facilities

(continued)

Activity	Address	Telephone
Applications Assistance Facilities	EROS Application Assistance Facility U.S. Geological Survey Rm. 202, Bldg. 3 345 Middlefield Rd. Menlo Park, CA 94025	415 323-2727
	EROS Applications Assistance Facility University of Alaska Geophysical Institute College, AK 99701 (Fairbanks)	907 479-7558
	EROS Applications Assistance Facility HQ. Inter-American Geodetic Survey Headquarters Bldg. Drawer 934 Fort Clayton, CZ	83-3897
Data Reference Files	EROS Data Reference File U.S. Geological Survey 5th Floor 80 Broad St. Boston, MA 02110	617 223-7202
	EROS Data Reference File Water Resources Division U.S. Geological Survey Rm. 343, Post Office and Court House Bldg. Albany, NY 12201	518 474-3107
	EROS Data Reference File State Topographic Office Lafayette Bldg., Koger Office Center Tallahassee, FL 32304	904 488-2168

(continued)

Table 2-2. Earth Resources Observation Services Data Centers and Facilities

(continued)

Activity	Address	Telephone
Data Reference Files	EROS Data Reference File Maps and Surveys Branch Tennessee Valley Authority 20 Honey Bldg. 311 Broad St. Chattanooga, TN 37401	615 755-2133
	EROS Data Reference File Topographic Office U.S. Geological Survey 900 Pine St. Rolla, MO 65401	314 364-3680
	EROS Data Reference File Water Resources Division U.S. Geological Survey 975 W. 3rd. Columbus, OH 43212	614 469-5353
	EROS Data Reference File Public Inquiries Office U.S. Geological Survey Rm. 7638, Federal Bldg. 300 N. Los Angeles, CA 90012	213 688-2850
	EROS Data Reference File Bureau of Land Management 729 N.E. Oregon St. Portland, OR 97208	503 234-3361
	EROS Data Reference File Public Inquiries Office U.S. Geological Survey Room 678, U.S. Court House Bldg. W. 920 Riverside Ave. Spokane, WA 99201	509 456-2524

(continued)

Table 2-2. Earth Resources Observation Services Data Centers and Facilities

Activity	Address	Telephone
Data Reference File	EROS Data Reference File Public Inquiries Office U.S. Geological Survey 108 Skyline Bldg. 508 2nd Ave. Anchorage, AK 99501	907 277-0577
	EROS Data Reference File University of Hawaii Department of Geography Rm. 313C, Physical Science Bldg. Honolulu, HI 96825	808 944-8463

(concluded)

(a) Attitude is the direction from which the photograph was taken. There are two attitude types: vertical and oblique. Vertical photographs are taken from directly overhead so that essentially you are looking directly down on objects, and oblique photographs are taken from an angle so that you see some of the side of the objects. For the purpose of the survey, the vertical attitude should be ordered. Oblique photographs can be used if vertical photographs are not available. However, their usefulness is limited.

(b) Scale is a measure of the ratio of the photograph to the ground area covered by the photograph. The basic film format is 23 square contimeters (9 square inches). Generally, each side of a photograph covers from between 5 to 14 kilometers (3 to 9 miles). You want as much detail as possible, therefore the smaller the ratio, the better. However, cost can be a factor, and it may be more economical to purchase photographs with a larger ratio and use the technique described in the next section to obtain the desired scale.

(c) Chromatism refers to the color of the photograph. Most older photographs are black and white. While many of the more recent photographs are in color or "false-color" a condition where, for example, vegetation may appear red due to the use of a film that is sensitive to a wavelength of light other than that in the visible spectrum. Generally speaking chromatism is not a concern and any color will be appropriate.

(d) Area is the location of the study. The latitude and longitude coordinates of the four corners of the area of interest should be supplied. A map outlining this area in red is also helpful.

(e) Intended use refers to stereoscopic or general. Stereoscopic coverage requires that each successive photograph overlap the prior one by a large margin. General coverage requires little overlap. Because the former requires more photographs to cover each area, it is more expensive. Therefore, general coverage is desireable.

(f) Time frame is the spacing in time between successive sets of photographs. In the past, most areas were not photographed very often. However, should coverage be frequent, photographs taken every 5 years are usually adequate.

(g) Index number refers to the number assigned to each photograph. Because of the large number of aerial photographs necessary to show an area on the ground, the photographs have been indexed by mounting a series of consecutive and adjacent overlapping photographs to create a mosaic of photographs of a specified area. These aerial photographic mosaics are referred to as "photo indexes" and allow for rapid identification of photographic coverage of a specific area. To order aerial

photography it is necessary that you initially order a photo index of your area of interest to determine the specific aerial photography needed.

Once you have received the photo index and identified the area of interest, put the project, roll, and frame number(s) on the order form which will come with the photo index.

Ordering takes time, sometimes as much as two months can elapse between your order for the photo indexes and the receipt of the individual photographs.

A priority system for rapid delivery of products is usually available whereby orders will be shipped within 5 working days of receipt. This process is very expensive however.

(3) Vegetative Mapping Using Aerial Photographs

Vegetative mapping is accomplished through opaque projection, outlined as follows:

(a) A blank sheet of white paper is secured to a drafting board or other suitable hard, flat surface.

(b) The board is positioned to serve as a screen to receive the projected image of the aerial photograph.

(c) The opaque projector is placed at a distance suitable to bring the projected image to the right scale. Once a given map size is established, all other photographs should be projected to the established map size.

(d) With a pencil, trace the boundary of all areas of interest, such as woodlots, fields, roads, and ponds. Also trace the boundaries of zones within these areas that represent different histories, such as different stages of growth in woodlots that may indicate different portions of the woodlots were cleared at different times. If in doubt, the team leader will determine what areas to trace. When the tracing is completed, use a lightboard to make a copy with permanent ink.

(e) Care should be taken to avoid damage to the aerial photographs from overheating. If cracks should appear on the projected image, remove the photographs and allow the projector to cool.

Once the maps are made, accurate measurements of area can be obtained with a polar planimeter (Figure 2-2) by tracing around the perimeter of the figure and reading the distance from the measuring wheel. The team leader will identify the areas to be measured. The following

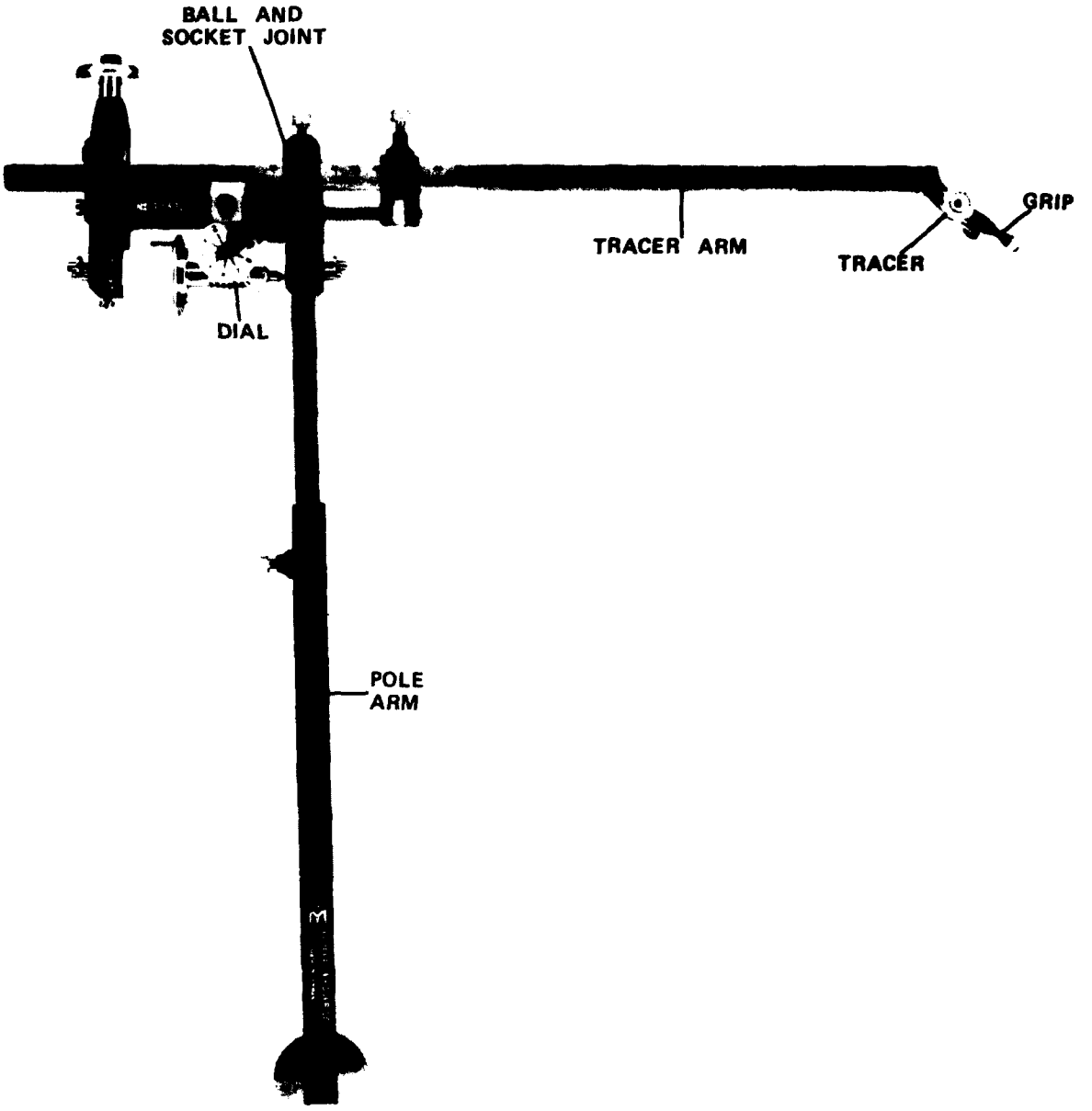


Figure 2-2. Polar Planimeter

steps are outlined in a manual for Compensating Polar Planimeters (Keuffel & Esser Co.). Make a rough preliminary circuit of the area and establish a starting (and finishing) point, then you are ready to make a measurement.

(a) Place the pole arm in the ball and socket joint.

(b) With the tracer point precisely at the starting point, take a reading from the dial, and write it down. Assume the reading is 3476 vernier units.

(c) With a firm hold on the tracer grip, move the tracer point around the outline of the figure carefully in a clockwise direction. As far as possible, the eye should be in line with the direction of the motion, and the tracer point should be close to the paper for precise work.

(d) If the tracer point inadvertently leaves the line it is not necessary to go back, but instead the tracer point may be intentionally moved the other way to compensate for the error.

(e) Do not attempt to use a guide for straight lines, as this is apt to produce a cumulative error. Straight lines can be traced with great accuracy by holding the head so the tracer point moves directly away from or toward the eye.

(f) When the figure has been circumscribed twice so that the tracer point has returned precisely to the starting point for the second time, take a second reading. Assume it is now 7491 vernier units.

(g) Place this number above the first reading and subtract the first from the second:

Second reading	7491
First reading	3476
Difference	4015

(h) If the 0 of the dial passes the pointer during the tracing, add 10,000 vernier units to the second reading. Thus:

Second reading	2765 = 12765
First reading	7452 = 7452
Difference	5313

The difference, 5313 is proportional to the area measured. The best way to determine the proportion is to identify two points on the map that are a known ground distance apart. For example, assume that two roads cross a third exactly one kilometer apart. Find the two roads on the map and make a square with the map distance between the roads on each side of the square. Use steps a through g above to measure the area of the square. For example, if the area of the square were 806, this represents the number of units in a square kilometer.[1] Therefore the area in question is:

$$\frac{\text{5313 units}}{\text{806 units per square kilometer}} = \text{6.6 square kilometers}$$

(4) Equipment Checklist Refer to Appendix F.

Photo indexes
Ordering forms (obtained from sources)
Aerial photographs
Blank white paper
Tacks or tape
Mounting board
Opaque projector
Ruler
Pencil and eraser
Lightboard
Planimeter

[1]Actually, 806 is the number of units in 2 square kilometers (you traced the perimeter twice). However, because you did the same with the unknown area, 806 is effectively the area of one square kilometer.

CHAPTER 3 - TREE RINGS: INDICATORS OF PAST SOIL POLLUTION

CONTENTS

a. APPLICATION

Tree ring analysis can be a useful tool in measuring past environmental stresses from pollution. Samples are collected and analyzed to determine: (1) if tree growth has been affected by pollution, (2) which year the pollution occurred and optionally (3) which pollutants were involved, and (4) the level of this pollution over time.

Ashby and Fritts inferred that tree growth was reduced due to past pollution[1]. It has been found that heavy metals are deposited in the annual growth rings of trees, where they remain as a permanent record of the ambient concentrations in the environment over time[2].

Generally, the concentrations of dissolved heavy metal ions in tree rings are influenced by the concentration in the substrate

[1]Ashby, W. C. and H. C. Fritts, "Tree Growth, Air Pollution, and Climate Near LaPorte, ID," Bulletin of the American Meteorological Society, 53:246-250, 1971.

[2]Rolfe, G. L., "Lead Distribution in Tree Rings," Forest Science, 20:283-286, 1974.

(e.g. soil and water if the tree were on a river bank)[1], rather than by those in the atmosphere. However, confirmatory tests of the soil/water may be desirable.

b. SAMPLING METHODOLOGY

(1) General

Identify the analytical laboratory which will perform the analysis. Heavy metal pollutants such as lead usually are analyzed by spectrophotometric dithizone extraction methods similar to those outlined by the Association of Official Agricultural Chemists[2]. Table 3-1 lists some of the laboratories working with tree ring analysis. Others may be identified by contacting the botany or chemistry departments of local universities. Tree rings can be measured by the paraecologist using the techniques below. However, it is desirable for experts to assist team leaders in interpreting the results.

Contact the analytical laboratory to determine if your collection procedures are adequate. Be sure that specimens are not rendered unsuitable for analysis by improper treatment.

Use a 5-millimeter (mm) Swedish increment borer to sample softwood (conifer) trees and hardwood (broad-leaved) trees. The environmental scientists on the team will select the specific trees to be sampled based upon criteria that identify trees with similar growth histories.

Before sampling each tree, borers must be cleaned with soapy water followed by xylene. At chest height, take a core perpendicular to the trunk toward the center of the tree. Be sure the core extends slightly beyond the center of the tree to ensure that all growth rings from bark to pith (the first year of growth) are included. Position the extractor spoon beneath the core and pull the extractor spoon (carrying the core) from the borer. Take two more core samples from the same side of the tree approximately 5 centimeters (2 inches) above and below the first. These samples will be used to determine which pollutants were involved and the concentration of the pollutant with time.

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[1]Sheppard, J. C. and W. C. Funk, "Trees as environmental sensors monitoring long-term heavy metal contamination of Spokane River, Idaho," Environmental Science and Technology, 9:638-642, 1975.

[2]Official Methods of Analysis of the Association of Official Agricultural Chemists, 10th edition, published by the Association of Official Agricultural Chemists, Washington, DC, 1965.

Table 3-1. Some Laboratories Performing Analysis of Tree Rings/Soils

Address	Chronology	Chemical Analysis of Wood	Chemical Analysis of Soils
ISOTOPES, A Teledyne Company Westwood Laboratories Westwood, NJ 07675		X	
Soils and Crop Department Rutgers University New Brunswick, NJ 08903		X	X
Department of Forestry and Institute for Environmental Studies University of Illinois Urbana, IL 61803		X	
Laboratory of Tree-Ring Research University of Arizona Tucson, AZ 85721	X		
Department of Chemical and Nuclear Engineering Washington State University Pullman, WA 99163		X	

Use the soil core sampler described on page E-2, Volume 6, to obtain soil samples. It may be necessary to obtain several samples from the same hole to ensure the soil is sampled to an adequate depth. Use the procedures described on page , Volume 5, to obtain water samples for chemical analysis.

(2) Record Keeping Refer to Appendix C.

Tree cores are numbered before complete removal from the borer. The number should consist of the identification number of the tree followed by "T", "C" or "B" for the top, center or bottom core, respectively. With a pencil[1], mark the tree identification (ID) number on the woody portion of the core next to the bark and if the core breaks, at the end nearest the bark. Following removal from the tree, place each sample in a drinking straw and label the straw with the sample number. In the event more samples are required, mark the tree with bright orange surveyor's flagging tied around the trunk at chest level. Mark the tree ID number of the tape with a permanent marking pen. Do not mark the numbers on the loose ends of the tape because they can become frayed.

Record pertinent data on the form shown in Figure 3-1. Tape the form to the log book.

The height of the crown (top of the tree) is obtained by measuring the angle (α) to the crown with a clinometer (α is entered on the space designated for clinometer reading) while you are standing 15.3 meters (50 feet) from the trunk of the tree (as measured by a cloth tape). Use the following formula to obtain the height to the crown (H) in meters:

$$H = 2 + \tan\alpha \cdot 15.3$$

Where 2 is the approximate vertical distance in meters from your eyes to the ground while you are taking the clinometer reading. The DBH (diameter, breast high) is measured with a DBH tape. At breast height, encircle the trunk with a DBH tape. Read the diameter directly from the tape.

The other entries are either obvious or will be discussed with you by the team leader.

(3) Refinement of Sample Refer to Appendix D.

The tree core samples can be refined by the paraecologist using

[1]Omit this step if it is suspected that the pencil lead will interfere with the chemical analysis.

ID Number _____ Soil Condition _____ Slope _____

Sun exposure_____ Associated Plant growth _____

Clinometer reading_____ Height of crown _____ DBH _____

Others _____

Chronology		Spectrophotometry		
Year	Ring Width (mm)	Chemical 1 level (ppm)	Chemical 2 level (ppm)	Chemical 3 level (ppm)

	Conc. in Water	Conc. in Soil					
		Depth	Level	Depth	Level	Depth	Level
Chemical 1	_____	_____	_____	_____	_____	_____	_____
Chemical 2	_____	_____	_____	_____	_____	_____	_____
Chemical 3	_____	_____	_____	_____	_____	_____	_____

Figure 3-1. Data Form for Tree Ring Analysis.

the techniques outlined in "preparation and examination of cores", pp 186-189 of Ferguson.[1]

Once the cores are prepared, the first step is to align the three cores from the same tree, and identify missing rings (those that are missing from one core but appear in the others) or false rings (caused by poor growing conditions in the middle of the growing season). It may also be necessary to check rings from other trees in the sample area to identify missing rings (see "statistical analysis", pp. 190-194 in Ferguson[1]).

Growth rate is measured with a scale loupe lens (10 power) fitted with a scale graduated in 0.1 mm increments. Measurements should be made on the core with the most complete series of growth rings, If all three are the same, measure the middle core. Starting with the first latewood (dark) cells aroung the pith and working toward the bark, measure the distance between successive latewood cells (annual growth rings), the first measurement is the second year of growth, the first year of growth cannot be accurately determined.

Tree core samples for chemical analysis will not be refined further; soil and water samples will be refined as per instructions from the receiving laboratory.

(4) Shipping

The straws containing the cores to be shipped should be attached to corrugated cardboard (one surface removed) with scotch tape. The ends of each straw should be pinched shut and taped. Prepare the samples for shipping as per the instructions for non-frozen material in Appendix E.

(5) Equipment Checklist Refer to Appendix F

Swedish increment borer (5-mm)
Soapy water
Xylene
Soda straws
Scotch tape
Corrugated cardboard
Mounting stick
Pencil
Permanent glue

(Continued)

[1]Ferguson, C. W., Concepts and Techniques of Dendrochronology, Reprinted from Chapter VII of Scientific Methods in Medieval Archeology, University of California Press, Berkeley, 1970. Distributed by Laboratory of Tree-Ring Research, University of Arizona, Tucson, AZ 85721.

Equipment Checklist (Continued)

Microtome, razor blade or belt sander
Compressed air jet
Cotton swabs
Kerosene
Scale loupe lens [7-15 power (10 is best)] with scale graduated in 0.1 mm
Common pins
Soil sampler (refer to Appendix E, Volume 6)
Bright orange surveyor's flagging
DBH tape
Cloth tape (feet/meter)
Clinometer
Data forms (Figure 3-1)

CHAPTER 4 - TURTLES: INDICATORS OF PAST AIRBORNE SOIL AND WATER POLLUTION

CONTENTS

a. APPLICATION

In circumstances where environmental effects from polluting activities are known or suspected to have occurred several years ago, there are not many biological indicators which would reflect past stresses. Many animal species are so highly vagile (mobile) that migration into previously stressed areas would mask past damage. For example, most mammal, bird, and fish species would quickly reoccupy depleted areas once a stress is removed. Other species (e.g., small rodents, amphibians, and invertebrates) have such high biotic potential (they reproduce so rapidly) that within a year or so after an environmental stress is removed, their populations would show no sign of that stress.

When trying to diagnose past environmental effects, turtle populations are ideal[1]. Turtles do not exhibit the shortcomings discussed above and they are found in sufficient numbers in most mesic (aquatic-terrestrial) habitats.

Two characteristics of turtle populations are usually of special interest in examining possible harmful effects of past polluting activities (1) ecological density and (2) population age distribution:

[1]The proper technique for handling turtles can be found in: Conant, R., A Field Guide to Reptiles and Amphibians, Peterson Field Guide Series, Houghton Mifflin Co., Boston, 1958.

(1) Ecological density is the number of turtles per unit of suitable area that can be colonized; in contrast to crude density which includes suitable plus non-suitable areas; e.g., "the number of box turtles in Hancock Township", even though the township contains homes, roads, parking lots and other areas uninhabitable for turtles.

If numbers of turtles in stressed areas are reduced, if immigration has not masked this reduction, and if not enough time has elapsed for the population to recover fully, then ecological densities of turtles in stressed areas should be lower than in non-stressed areas.

(2) Population age distributions are the numbers of individuals of given age groups in the population. Age distributions of turtle populations in stressed areas could also be quite different from those in non-stressed areas.

Non-stressed areas would probably harbor populations with the density determined by the environmental carrying capacity (this is the upper level of population density beyond which no major increase can occur). A static turtle population at the carrying capacity of the environment has a fixed density based on the area of suitable habitat. It is a group in which numbers of births and deaths cancel each other on the average, and the population is characterized by an unchanging age structure.

In contrast, a population below the carrying capacity exhibits other predictable characteristics. Slobodkin[1] discussed this circumstance:

> "Let us imagine a population that is suddenly presented with the opportunity for exponential increase. Initially, the number of animals in the first age category will increase, while the number of animals in the older age categories will remain the same as when the population was static. Little by little all of the age categories will show an increase as the survivors from the enlarged initial category grow older. As more animals enter the reproductive categories more and more newborn will be produced."

Therefore, if a turtle population had been stressed years ago, but if the stress had been removed and the population had begun a recovery, the rapidly expanding group would initially contain a large proportion of young individuals. There would be a shift in age distribution toward young age groups.

To recapitulate, there should be fewer turtles per acre of suitable habitat in stressed versus non-stressed populations. If the stress occurred long ago, a recovering population should have a higher-than-normal proportion of young individuals.

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[1]Slobodkin, L.B., Growth and Regulation of Animal Populations, Holt, Rienhart and Winston, Inc., 1964.

b. SAMPLING METHODOLOGY

(1) General

To measure ecological density and age distribution, turtles must be captured and worked with individually. Many species can simply be collected by hand, while walking systematically through suitable habitats during the right time of year (many species hibernate in winter) and during the right time of day (some species, for example, bask early in the morning).

Pit traps along 15-centimeter (6-inch) high drift fences are often successful in capturing small turtles moving on land. Drift fences[1] consist of nothing more than a length [usually at least 27 meters (90 feet)] of 1 centimeter mesh hardware cloth strung flush with the ground between support posts. Sink number 10 vegetable cans into the soil flush with the surface, at about 9-meter (30-feet) intervals along both sides of the drift fence. When a wandering turtle encounters the fence, he crawls left or right following the fence until he falls into a trap. Check the drift fence daily.

(2) Record Keeping Refer to Appendix C.

In the field, place a length of masking tape on the carapace (upper shell) of the turtle and mark an identification number on the tape with a permanent ink, felt tip pen. To avoid being scratched and bitten, it is best to grab the upper shell slightly toward the front. In addition to the number, include the names of the collectors, the habitat type and the date. Mark the ID number over the collection location on your map. Place the turtle in a cloth bag.

Turtles can be permanently, harmlessly, and individually marked by filing (Figure 4-1) or grinding (Figure 4-2) notches into marginal scutes according to a coded combination (Figure 4-3). Youngsters with pliable shells can be marked using fingernail clippers.

At each initial capture and recapture, record the appropriate data on data sheets (Figure 4-4). Measure the carapace length and width with calipers. Special remarks can be recorded such as laying eggs, basking, fighting, found dead. The "Map of the Study Area" and Illustration" should be part of the original data sheet. The location of capture or recapture will be placed on the map. The illustration consists of a dorsal and ventral diagram of a typical carapace and plastron (lower shell) of the species being studied. Marking notches, natural deformities and unusual markings can be drawn on these illustrations

[1]Refer to Figure 8-2, Chapter 8.

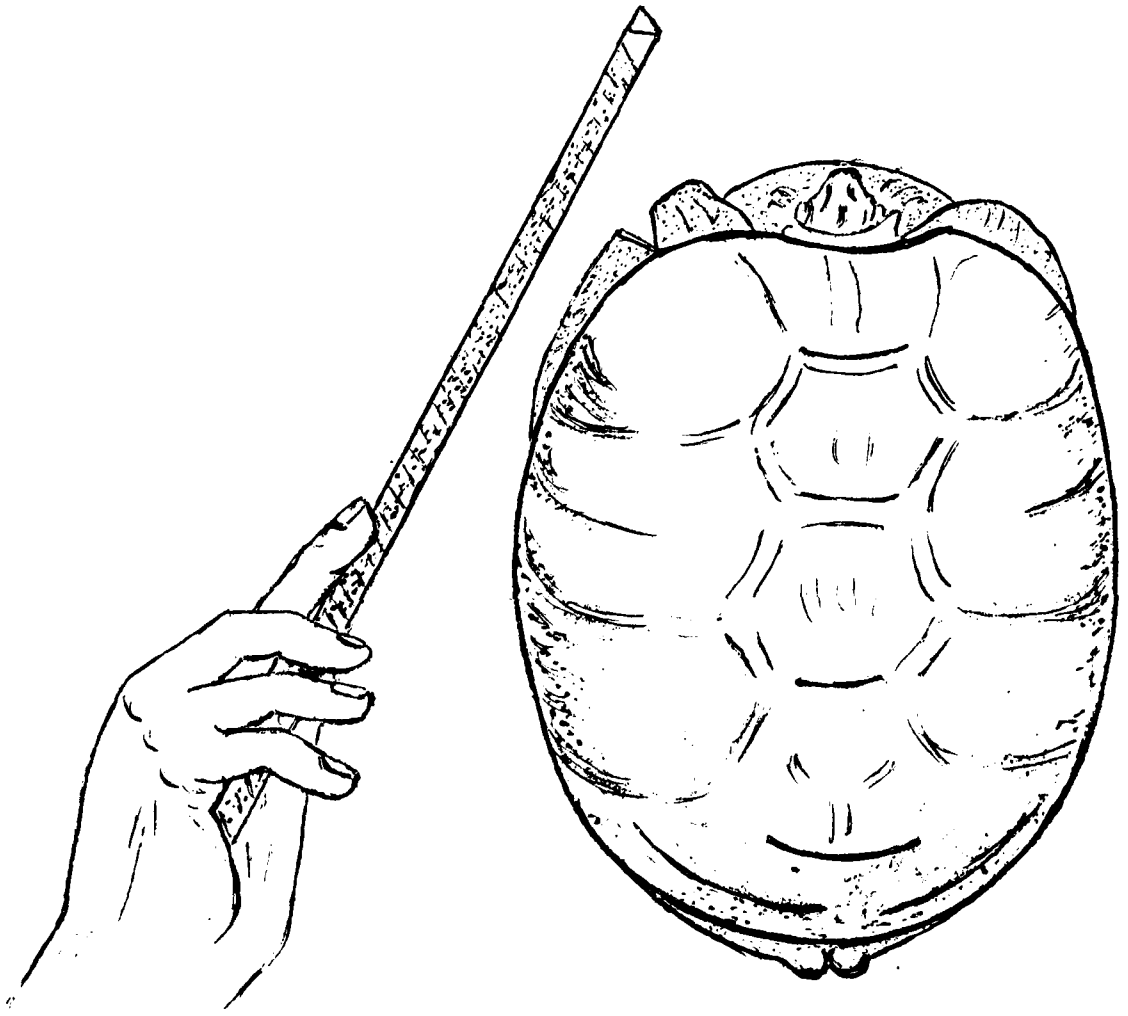

Figure 4-1. Filing Notches Into Marginal Scutes

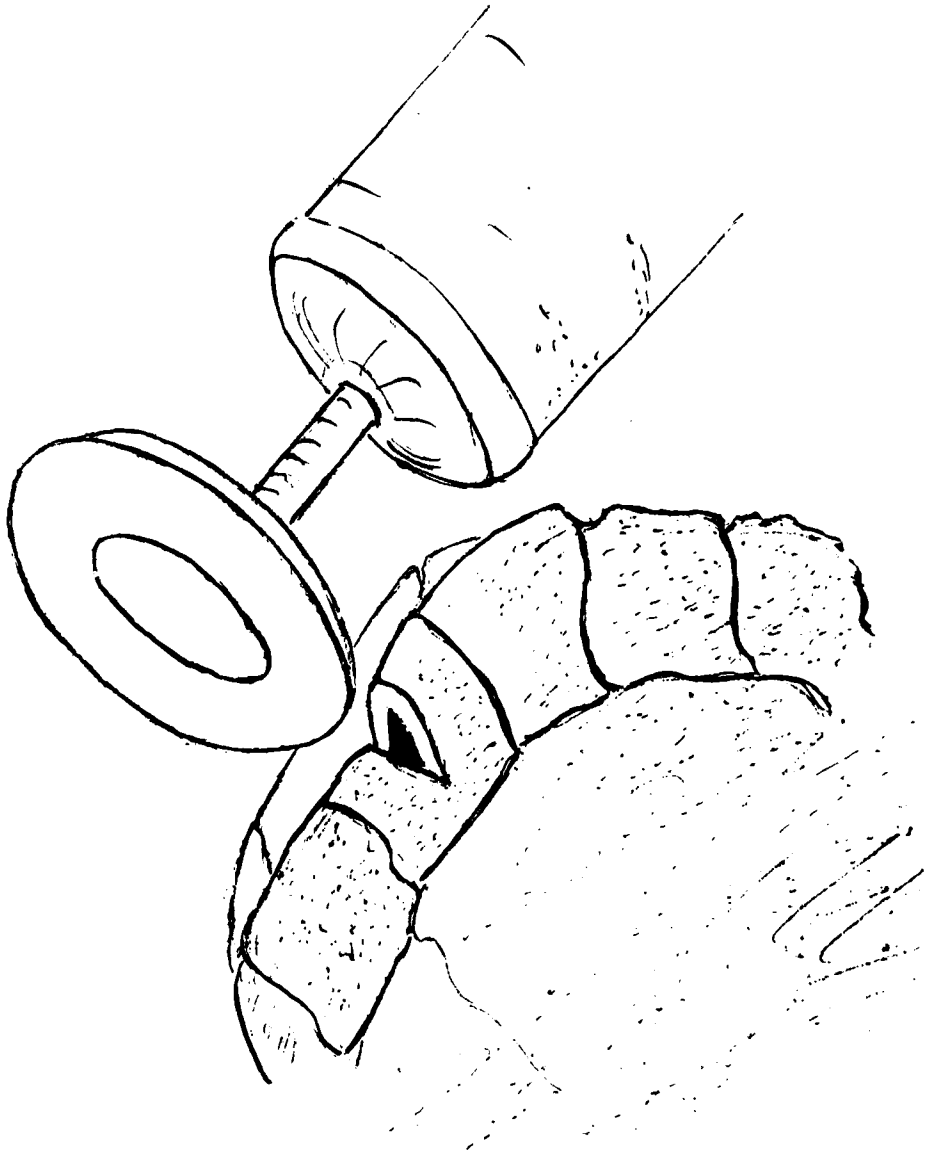

Figure 4-2. Grinding Notches Into Marginal Scutes

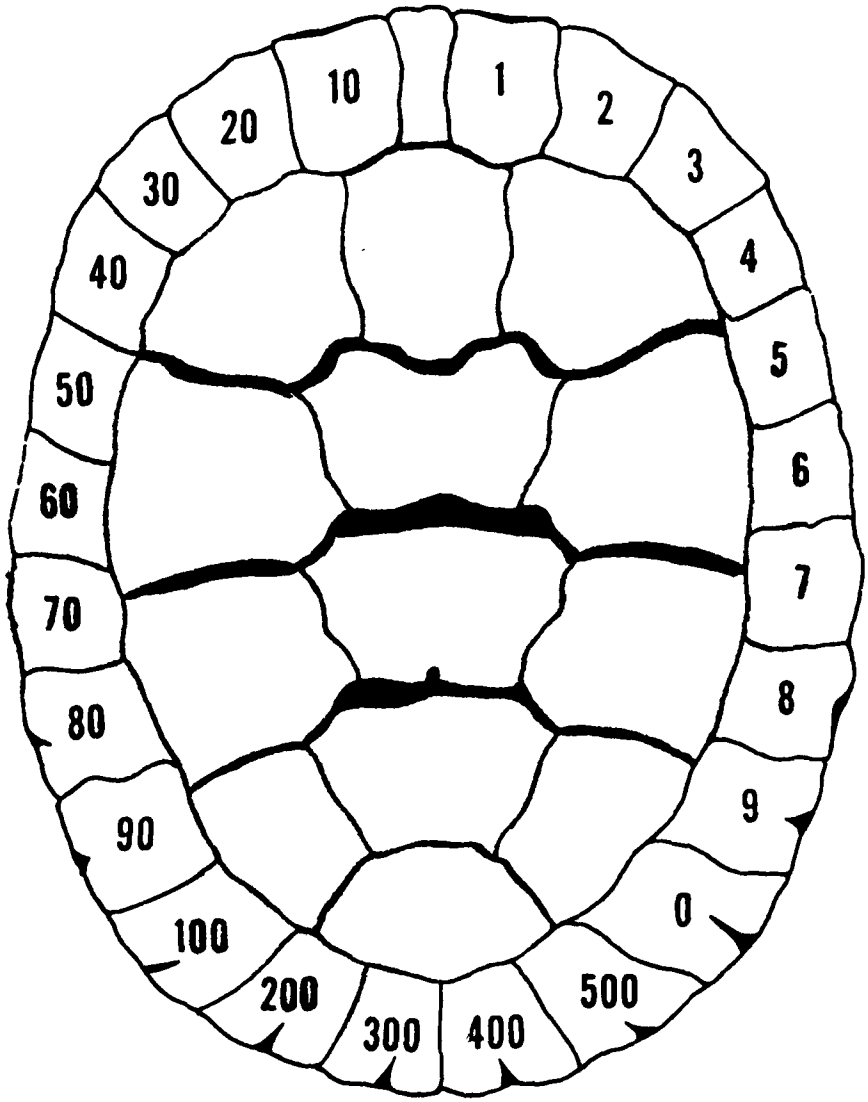

Figure 4-3. Coded System for Numbering Turtles

Species _____

Number _____

Capture Location _____

"Map of Study Area"

Capture Date _____

Release Date _____

Remarks _____

Sex _____

Weight (gm) _____

"Illustration"

Carapace Length (mm) _____

Carapace Width (mm) _____

RECAPTURE DATA						
Recapture Date	Release Date	Location	Wt	L	W	Remarks

Figure 4-4. Turtle Data Sheet

as a double check for proper identification or subsequent captures. The remainder of the entries are self-evident.

Probably the best reference book on turtle habits, ranges, identification and aging and sexing of individuals is Turtles of the United States.[1]

(3) Data Analysis

A general idea of population size can be obtained by marking as many turtles as possible during one year, then sampling again the following year:

$$\frac{\text{Number Marked in Year 1}}{\text{Total Population}} = \frac{\text{Number Marked Turtles Caught in Year 2}}{\text{Total Number Caught in Year 2}}$$

For example, if 100 turtles of a given species were marked during the first year and 200 were caught in the second year of which 25 had been marked the year before:

$$\frac{100}{\text{Total Population}} = \frac{25}{200}$$

$$\text{Total Population} = \frac{(100)\ (200)}{25} = 800$$

This figure can then be divided by the total acres of suitable habitat to determine the number of turtles per acre, i.e., the ecological density.

Determining actual age distributions is more difficult because young (small) turtles are usually much more secretive than adults, thus they are less likely to be captured. However, a perfectly suitable index of age distribution can be obtained by comparing the proportion of young turtles in the total population caught in an experimental area with the proportion caught in a reference area. Because the catchability of youngsters in both areas should be the same, inferences (e.g., "There are probably twice as many immature turtles in Area A versus Area B") should be valid even though absolute numbers are not known.

[1]Ernst, C. H. and R. W. Barbour, Turtles of the United States, University Press of Kentucky, 1972.

(4) Equipment Checklist Refer to Appendix F.

15-cm high drift fence
Vertical support posts
Number 10 vegetable cans
Shovel
Masking tape
Permanent ink felt-tip pen
Cloth bags
Triangular file
Grinder
Fingernail clippers
Calipers

CHAPTER 5 - FOLIAGE CONDITION: INDICATOR OF PRESENT AND FUTURE AIRBORNE POLLUTION

CONTENTS

a. APPLICATION

Injury to vegetation caused by exposure to air pollutants has provided a major indicator for evaluating the effects of polluting activities on the environment.[1] However, recognition of pollutant-induced effects and identification of the specific causal agents may be difficult when the plant injury is caused by more than one pollutant emanating from a single source or from different sources. Furthermore, there may be an additive effect on plant injury when more than one pollutant is emanating from a single source.

Plants vary greatly in their sensitivity to various air pollutants. It is necessary, therefore, to have knowledge about the type of pollutant present, its concentration and its distribution with time i.e. hour, day, week and month, to note the kind and extent of plant injury and to compile a list of resident plants as to their degree of resistance or susceptibility to the specific air pollutant.

Many pollutants will bioaccumulate in leaves, therefore, chemical analysis of leaves may be used to identify the pollutant causing the leaf damage.

[1]Jacobson, J. S. and A. C. Hill, Recognition of Air Pollution Injury to Vegetation: A Pictorial Atlas, Air Pollution Control Association, Pittsburgh, PA, 1970.

b. SAMPLING METHODOLOGY

(1) Air Pollutant Source Survey

The sources and identities of all air pollutants capable of affecting the study area should be obtained using the techniques of Volumes 2 and 3. Procedures for meteorology and model development may be obtained from Volumes 3 and 7.

(2) Field Surveys of Foliage Injury

General

The first step in evaluating foliage injury is to make a preliminary inventory of the survey area identifying plant species (refer to Appendix A), their abundance and distribution and their economic, ornamental, esthetic or protected status. Use the techniques of Section A.2.e.(2), Volume 2 and pages 2-34, the Navy's inventory manual[1], followed by Chapters 1 and 2 of this volume.

As suggested by Cole[2], the plants selected for indicator species should: (1) respond to lower concentrations of the pollutants than the economic, ornamental, esthetic or protected plants, (2) be widely distributed, (3) exhibit easily identifiable pollutant induced injury, (4) be present throughout the growing season, and (5) grow from a terminal shoot throughout the growing season because new growth is most susceptible.

The susceptible plants that have been selected for survey should be examined carefully for foliar injury. The use of a pictorial atlas such as that published by the Air Pollution Control Association[3] should be used to evaluate the pollutant effect.

Initial scientific supervision should minimize confounding foliar injury attributable to pollutants with that caused by insect bites, drought, physical injury, nutrient status and senescence.

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[1]U.S. Department of the Navy, Naval Facilities Engineering Command, Alexandria, VA 22332. Study of Ecological Classification and Inventory Manual, M. M. Goodwin, Oct 1977.

[2]Cole, G. A., "Air Pollution With Relation to Agronomic Crops, III. Vegetation Survey Methods in Air Pollution Studies," Agronomy Journal, 50:553-555, 1958.

[3]Jacobson, J. S. and A. C. Hill, Recognition of Air Pollution Injury to Vegetation: A Pictorial Atlas, Air Pollution Control Association, Pittsburg, PA, 1970.

Record Keeping

Mark each sample site where sensitive species are growing with a lath stake to which a luminescent orange plastic ribbon is securely tied. Areas selected must be accessible and be charted on a map. Effects due to pollution should be distinguishable from other local effects thus a sufficient number of indicator plants should be located in the experimental area to give statistically significant results.

If the plant is not too large, the complete above-ground part of the plant should be photographed and collected, otherwise a sprig or branch will suffice. Photograph the plant (in the field) on a black background with a good single lens reflex camera with microfocus capability. A good quality color film should be used to record the plant appearance. Include standard label (Appendix C) in the photograph of the injured plant to document pertinent data about the sample. Record the cause of the foliar injury under "conditions" on the standard label.

Following the photographing of the plant, place it in a portfolio[1] and transport it to your laboratory work area. The field portfolio may consist of a number of folded sheets of paper (46 by 61 centimeters, 18 by 24 inches) or newspapers folded to a rectangle (30 by 46 centimeters, 12 by 18 inches). The sheets of paper with the plants and standard label sandwiched in between are placed along with other sheets containing plants and standard labels between two pieces of heavy cardboard or pieces of one quarter inch plywood and strapped with a web belt using a friction buckle to hold the press in a compressed form.

When you return to your laboratory work area, transfer the plants to a plant press[1] as soon as possible. The plant press is constructed in a manner similar to the portfolio. Each folded paper, containing a plant specimen and its label, is placed between two sheets of heavy felt paper driers, 30 by 46 centimeters in size. These in turn are enclosed between corrugated cardboard sheets of the same size to facilitate drying from circulating air. The above arrangements are then placed one on top of the other and strapped together between two slat frames. After drying for 24 to 30 hours the moist felt driers are removed and replaced with dry felts and again they are pressed as tightly as possible and placed in a warm dry place until completely dry. When drying is completed, the plants are removed from the press and attached to sheets of stiff white paper with white binding tape. Glue the standard label in the lower right hand corner.

[1]U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705. Preparing Herbarium Specimens of Vascular Plants, C. E. Smith, Jr., Sep 1971.

(3) Chemical Analysis of Vegetation

General

Chemical analysis of vegetation serves two purposes: (1) to monitor the accumulation of toxic or potentially toxic pollutants in vegetation which is consumed by humans or animals, and (2) to furnish a general and relative index of pollution. The former usage is much more important, however, since air monitoring analysis is more accurate and economical than plant analysis.

As there is concern with the toxic effects of the plant-borne pollutant on humans or livestock, sample only those portions of the plant that would be ingested. For example, if one were sampling sweet corn (Zea mays), only the kernels would be collected. If the corn were to be ensiled for cattle feed then the whole plant above 10 centimeters (4 inches) would be collected. Dry the sample in a plant press as above.

Record Keeping

A standard label (Appendix C) should be completed for each sample.

(4) Refinement and Shipping of Sample

Prepare the dried plant or sample for shipping by placing it in a plastic bag (or other suitable container specified by the receiving laboratory) along with a reproduced copy of the standard label. Ship it to the receiving laboratory by the method for shipping non-frozen samples in Appendix E.

(5) Equipment Checklist

30 x 46 cm wooden frame covered with black velvet stretched over the frame and secured with carpet tacks
Sharp pocket knife
White paper tape
Drying portfolio equipped with a web belt and friction buckle
Copy of Recognition of Air Pollution Injury to Vegetation: A Pictorial Atlas
Copy of Preparing Herbarium Specimens of Vascular Plants
Plant press with web belt and friction buckle
Felt drier pieces 30 x 46 cm
White cards 30 x 46 cm
Mounting tape
Pruning shears

CHAPTER 6 - EARTHWORMS: INDICATORS OF PRESENT AND FUTURE SOIL POLLUTION

CONTENTS

a. APPLICATION

Earthworms are widely distributed in nature and according to Clark[1] may be the dominant animal life in the soil. They flourish best in well-drained soils that contain abundant organic matter and a continuous supply of available calcium. Earthworms number several million to an acre in favorable soils. Their total weight in an acre of fertile soil may be 1/2 ton. However, they are susceptible to drought, cold, waterlogging and extremes of acidity or alkalinity.

Earthworms may serve as effective monitors of environmental pollution because, "these invertebrates may exert a significant influence on the distribution of trace elements in soils and food chains by altering concentration in tissues through bioaccumulation."[2] Ireland[3] indicated that earthworms in soils with high levels of iron, zinc and

[1]U.S. Department of Agriculture, Washington, DC 20250, "Living Organisms in the Soil", Yearbook of Agriculture, Soils, F. E. Clark, 1957.

[2]Van Hook, R. I., "Cadmium, Lead and Zinc Distributions Between Earthworms and Soils; Potentials for Biological Accumulation", Bulletin of Environmental Contamination and Toxicology, 12:509-511, 1974.

[3]Ireland, M. P., "Metal Content of Dendrobaena rubida (Oligochaeta) in a Base Metal Mining Area", OILOS, 26:74-79, 1975.

lead retained high levels of lead within their tissues, and Gish[1] reported that concentrations of organochlorine residues such as DDT occurred in earthworms at concentrations up to nine times that found in their native soil. Edwards and Jeffs[2] suggested a linear correlation between the concentration of DDT and other organochlorine insecticides in earthworms and corresponding soils, but not all earthworm species concentrate insecticides in the same way nor to the same degree. Dimond et al[3] found DDT in soil with higher levels in earthworms and the highest levels in robins which feed on earthworms.

b. SAMPLING METHODOLOGY

(1) General

The large number of specimens required for a chemical analysis suggests the use of an expellant as the method of choice for collection. Raw[4] reported the superiority of formalin as an expellant to bring worms to the surface where they could be collected readily. Care should be taken due to the toxic nature of formalin. Fumes should be avoided as much as possible and the person(s) applying the solution should be indoctrinated to its dangers.

The expellant solution is prepared by adding 50 milliliters of 40% formalin to 4 liters of water. Apply one liter of solution to each 30 centimeter square (1 square foot) area of soil surface. The soil surface is prepared by removing all soil litter and debris. Apply the dilute formalin to the soil with a watering can or similar sprinkling device. Next saturate the soil surface with water to wash the dilute formalin into the soil.

Within 5 to 20 minutes earthworms will surface and be available for collection. Specimens should be washed with clean water to free them of extraneous material and placed in petri dishes containing water-moistened filter paper. This method is most effective in the spring and autumn when the soil temperature and moisture conditions

[1]Gish, C., "Organochlorine Insecticide Residue in Soils and Soil Invertebrates from Agricultural Lands", Pesticide Monitoring Journal, 3:241-252, 1970.

[2]Edwards, C. A. and K. Jeffs, "Rate of Uptake of DDT from Soil and Earthworms", Nature, 247:157-158, 1974.

[3]Dimond, J. B., G. Y. Belyea, R. E. Kadunce, A. S. Getchell and J. A. Blease, "DDT Residues in Robins and Earthworms Associated with Contaminated Forest Soils", Canadian Entomology, 102:1122-1130, 1970.

[4]Raw, F., "Estimating Earthworm Populations by Using Formalin", Nature, 184:1661-1662, 1959.

are such that worms are active close to the surface. Information on the specific effects of soil temperature, moisture content and time of year on the harvesting of earthworms can be found in Soil Biology[1].

(2) Record Keeping

All earthworms of a single species collected from a square meter plot can be recorded on a standard label (Appendix C) and that label attached to the petri dish. Species can be identified using Earthworms of Ontario[2], also refer to Appendix A.

(3) Refinement of Sample

Many methods are available for chemical analysis, including gas chromatography, gas-liquid chromatography, spark source mass spectrometry, and atomic absorption spectrophotometry. Most large universities can perform these analyses. Prior to shipping, earthworms will be frozen by placing all the petri dishes with standard labels in a plastic bag in a -21° Celsius (-6° Fahrenheit) freezer for several days.

(4) Shipping

Ship the bags containing the earthworms by the method for frozen samples in Appendix E.

(5) Equipment Checklist Refer to Appendix F.

Formalin (40%)
Clean water (adequate quantity to formulate expellant, saturate soil, wash specimens, and retain moisture in holding containers)
Containers for holding and dispensing the clean water
Container for mixing formalin solution (4 ℓ, 1 gal)
Container for sprinkling formalin solution (one ℓ, 1/4 gal)
Petri dishes and moist filter paper
Plastic bags

[1]Burgess, A. and F. Raw, Soil Biology, Academic Press, London and New York, 1967.

[2]Reynolds, J., The Earthworms of Ontario, Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario M5S2C6, 1977.

CHAPTER 7 - BEES: INDICATORS OF PRESENT AND FUTURE AIRBORNE POLLUTION

CONTENTS

a. APPLICATION

The economic importance of bees cannot be overestimated. Bees are required for the pollination of one third of our total food supply[1]. Without them, human and wildlife populations would be compelled to eat mostly grasses and some species of nuts.

Bees make excellent biological indicators for present and proposed activities for the following reasons:

(1) Certain groups are common.

(2) Bees have a low tolerance to most pesticides and certain heavy metals. However, the degree of tolerance varies with the pollutant and some pollutants immediately knock down bees in the field while others kill slowly in the hive, necessitating different techniques discussed later. At the same time bees can bioaccumulate radioactive, industrial

[1]U.S. Department of Agriculture, Agricultural Research Service, Washington, DC 20250. "Insect Pollination of Cultivated Crop Plants", Agricultural Handbook Number 496, S. E. McGregor, 1976.

and automotive pollutants in the pollen and honey stored in the hives.[1,2,3]

(3) Bees are short-lived and have a high biotic potential (reproduce rapidly). Therefore, changes in population structure would be useful in determining the efficacy of pollution abatement measures. However, honey can be stored for periods longer than one year, therefore, honey should be analyzed with caution for this purpose.

(4) Pollen surveys can indicate species of plants selected, and potentially, thereby, the area where the bees are foraging. This could enable the team to localize the areas of highest stress.

(5) Bees can be used as sentinel species by attracting them to various artificial living quarters.

The four groups that are common enough for our purposes are:

Honey bees (Family Apidae). Since honey bees are a "domestic" insect, they may be subjected to manipulation and detailed data gathering processes described later. Honey bees, unlike most wild pollinators, collect nectar or pollen or both from a wide assortment of plants. There are few crops, whose blossoms are attractive to any insect, that do not hold some attraction for honey bees.

Alkali bees (Family Halictidae). The alkali bee is about the size of a honey bee with bright green metallic markings on the upper abdomen. The alkali bee is important as a pollinator of alfalfa west of the eastern escarpment of the Rocky Mountains. It pollinates alfalfa more efficiently than the honey bee which prefers to obtain nectar rather than pollen, leaving the blossom unpollinated. It nests together with other alkali bees in alkaline soil, each bee having its own nest with a small mound marking the entrance.

Leafcutter bees (Family Megachilidae). They are stout-bodied, dark-colored bees that carry pollen on a distinctive brush of hairs

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[1]Hakonson, T. E. and K. V. Bostick, "The Use of Honey Bee Colonies as Bioindicators of Cesium 137, Tritium and Plutonium in the Los Alamos Environs", 19th Annual Meeting of the Health Physics Society, 27:632, 1970.

[2]Tong, S., R. A. Morse, C. A. Bache and D. S. Lisn, "Elemental Analysis of Honey as an Indicator of Pollution," Archives of Environmental Health, 30:329-332, 1975.

[3]Webster, B., "Honeybees Aiding Pollution Fight," New York Times, p. L 41, 24 Sep 1975.

on the ventral side of the abdomen. These common bees nest in the ground or in a natural cavity. Cell partitions are of leaf pulp, mud or resin. A few nest with other species of bees.

"Orchard bees" (several species in several families including the three families discussed above - not a valid taxonomic grouping). When fruit trees are in blossom, bees of all kinds will suddenly appear in great abundance. Bees foraging at fruit trees at this time may be sampled to screen for the presence of pollutants from a wide area.

Other considerations in their use are that:

(1) With the other biological indicators selected, bees may die suddenly from a number of causes not related to pollution stress: disease, parasites, predators, starvation, lack of water, and extreme weather changes. Old age eventually claims bees, but in a more orderly manner than a catastrophe.

(2) Many pesticides will knock down bees quickly, and they die in the field, never returning to the hive or nest where deaths may be more conveniently counted. Even moderate reduction of adults may cause starvation, killing off the entire hive, thus obscuring the actual effect of a pesticide. In a recent book McGregor has an in-depth discussion on the effects of pesticides on bees.[1]

(3) Bees are highly seasonal in their foraging activity. In the northern half of the United States, honey bees are active for approximately 5 months outside the hive and some wild bees for less than 2 months, depending upon species. Activity increases gradually as one heads southward until in the extreme southern United States bees are active year round.

(4) Bees account for many more human deaths than do all the poisonous reptiles combined. As such, bees should be handled with caution by non-sensitive personnel properly trained in correct handling techniques.

b. SAMPLING TECHNIQUES

(1) General

Determine that sufficient numbers of bees for sampling are present in the experimental and reference areas by looking for them around

[1]U.S. Department of Agriculture, Agricultural Research Service, Washington, DC 20250. "Insect Pollination of Cultivated Crop Plants", Agricultural Handbook Number 496, S. E. McGregor, 1976.

blossoms of clover, alfalfa, fruit trees and other flowering plants.

If it is decided to analyze bees for pollutants, the bees can be collected by using sweep nets. Kill the bees in a jar partially filled with dry ice. Another way to trap bees is to attract them to nesting structures placed where water and food are already available.

Hives[1] of honey bees are transportable and can be placed in experimental and reference areas prior to releases of pollutants; or can be placed in areas where pollutants have already been released. The hives can then be censused for unusual death rates, or the bees, their stored honey or pollen can be analyzed for the presence of pollutants.

Waller[2] developed a mini-hive consisting of (a) a small styrofoam picnic chest or "six-pack cooler", (b) cove molding, used for the foundation of combs, (c) a 2.5 centimeter (1 inch) hole near the bottom at one end, which serves as the entrance, and (d) a brick on the lid to hold down the box.[3]

Semi-weekly inspection is required because the small size allows little margin between starvation and overcrowding. Also bees will sometimes chew through the styrofoam. Some researchers prefer the mini-hives because they are inexpensive and easily started with a queen and a few bees.

Hive Counts of Dead Honey Bees

Most foraging bees normally die in the field after about 6 weeks of activity.[4] Therefore, a large number of dead bees near the hive would be of concern. Two types of pollution could cause premature bee mortality:

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[1]U.S. Department of Agriculture, Agricultural Research Service, Washington, DC 20250. "Beekeeping in the United States", Agricultural Handbook Number 335, 1971.

[2]Waller, G. D., "Styrofoam Mini-hives", Gleanings in Bee Culture, 105:344-345, 1977.

[3]Dr. Roy J. Barker, U.S. Department of Agriculture, Bee Research Laboratory, Tucson, AZ 85719. Personal communication, has informed the author or an advanced design mini-hive from a plastisized paper box with frames fit inside, manufactured by Sundance of Santa Ana, CA.

[4]Gary, N. E., "A Trap to Quantitatively Recover Dead and Abnormal Honey Bees From the Hive", Journal of Economic Entomology, 53:782-785, 1960.

(1) Some pollutants will kill the bees in the field. If the field force is sufficiently reduced, the entire colony may die of starvation.

(2) On the other hand, bees exposed to certain pollutants (especially stomach poisons) may be able to return to the hive with contaminated food, especially pollen. Before death they can introduce poison into the entire hive, including larvae and the queen.

The simplest way to obtain counts of dead bees at the hive is to clear an area with a hoe or shovel about 2 meters square in front of the hive. Housecleaning bees remove dead bees from the hive and normally drop their bodies to the ground around the entrance. Dead bees should be counted and removed from the cleared area at the same time each day.

The count from the above method is, at best, qualitative because scavengers remove carcasses or some dead bees may be missed. When a more precise method of counting dead bees is required, hive entrance traps are used. A typical entrance trap is a galvanized sheet metal box about the size of a standard deep 10-frame hive body or super[1]. The trap is secured to the hive at the entrance with 4 penny nails. The heads of the nails are left protruding so they can be pulled easily. The bottom consists of tilted louvers 4 millimeters (3/8 inch) apart, through which housecleaning bees fly out after carrying disabled or dead bees into a tray beneath. A recent modification is shown in Figures 7-1 through 7-3[2].

Chemical Analysis of Honey Bees, Honey and Pollen

Dead bee carcasses from dead bee traps can be pooled for chemical analysis when toxic organic compounds are involved. Because of possible contamination from the soil, do not use dead bees collected from the ground in front of the hive.

If the pollutant is a trace element or radionuclide, generally use live bees caught at the entrance to the hive. Free-flying honey bees may be collected at the hive with a suction apparatus.

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[1]A super is a portion of the hive body used in the production of extracted honey. It is of wood with dovetailed corners and the inner top edge rabbeted out for a close fit with the next upper section of the hive.

[2]Commercially available from Dr. Adair Stoner, Research Entomologist, Honey Bee Pesticides/Diseases Research University Station, Box 3168, Laramie, WY 82071.

Figure 7-1. Modified Dead Bee Trap Attached to Hive Entrance

Figure 7-2. Dead Bee Trap From Above With Double Hardware Cloth Mesh Removed (Lifts Out), Showing Metal Louvers, Below Which the Dead Bees Are Deposited Into a Collecting Drawer

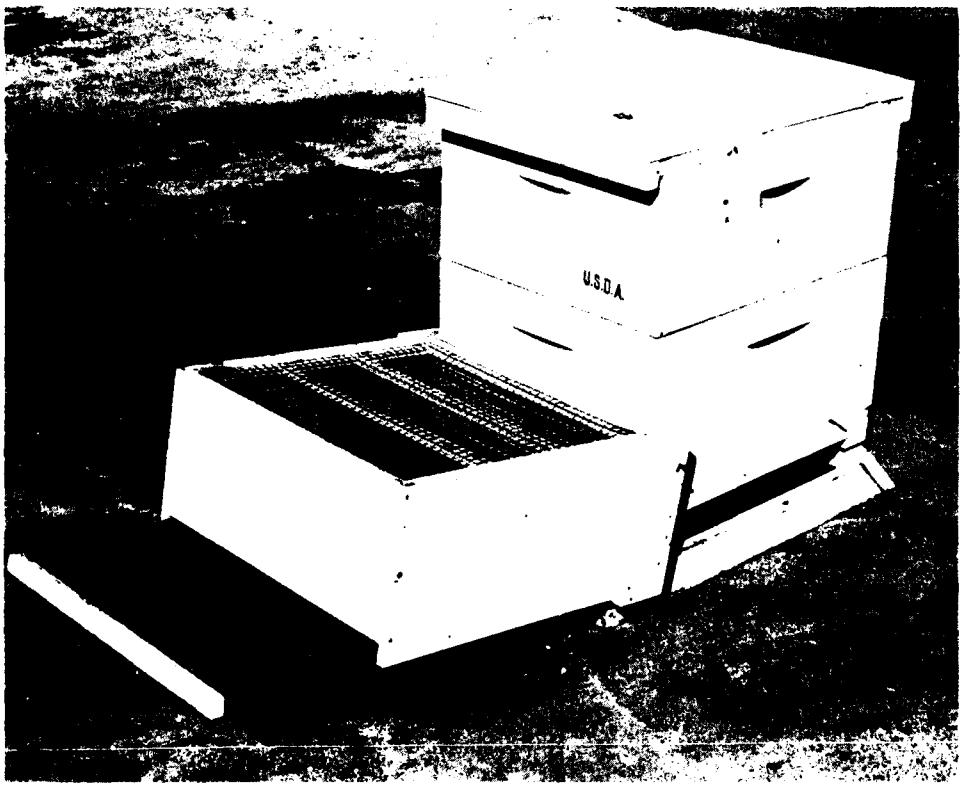

Figure 7-3. Dead Bee Trap With Collecting Drawer Pulled Out

Bromenshenk[1] has developed a 12-volt battery-powered aspirator suitable for collecting adult bees (Figure 7-4). A 12-volt battery powered squirrel cage fan provides a vacuum and is connected to the exhaust outlet via a flexible plastic hose. Air and bees are drawn through the venturi inlet; the bees are deposited in the collection jar which contains dry ice. The jar is removed by unscrewing it from the aspirator, giving a sharp downward rap to force the bees to the bottom, and quickly removing and capping the jar. The sample size per pool will be determined by the team leader.

When removing honey or pollen from the hive[2] use a wood or porcelain spatula to uncap cells and remove honey. Mechanical honey extractors may introduce metal contamination. Cut out a small comb portion of newly stored nectar and/or honey from the super and place in vial.

Wild Bees

These bees serve as pollenizers of alfalfa and other crops such as onions as well as many native plants. The greatest advantage of using wild bees as biological indicators is their chance presence when observation and data collection must be made without availability of baseline data. There may be species differences in sensitivity. Smaller bees such as alkali or leafcutter bees are affected adversely more than honeybees and bumble bees by some insecticides (e.g. Carbaryl, Orthene and Methonyl)[3].

Devices which attract and foster the propagation of bees and wasps that nest above ground also may serve as instruments for collecting specimens and estimating population dynamics. One such device is made from a drilled bee board[4] (Figure 7-5). The back measures 15 by

[1]U.S. Environmental Protection Agency, Corvallis, OR 97330, "Investigation of the Impact of Coal-fired Power Plant Emissions upon Insects: Entomological Studies in the Vicinity of Colstrip, MT", J. J. Bromenshenk, pp 140-212, In: E. M. Preston and R. A. Lewis (eds), The Bioenvironmental Impact of a Coal-fired Power Plant, Third Interim Report, Colstrip, MT, Dec 1977, 1978.

[2]When organic pollutants are involved, do not use the wood burning smoker customarily used to sedate bees, rather use a distilled water mist.

[3]Johansen, C. A., "Toxicity of Field-weathered Insecticide Residues to Four Kinds of Bees", Environmental Entomology, 1:393-394, 1972.

[4]Bee board commercially available from Leonard Tiegs, Box 2524 Nampa, ID 83654.

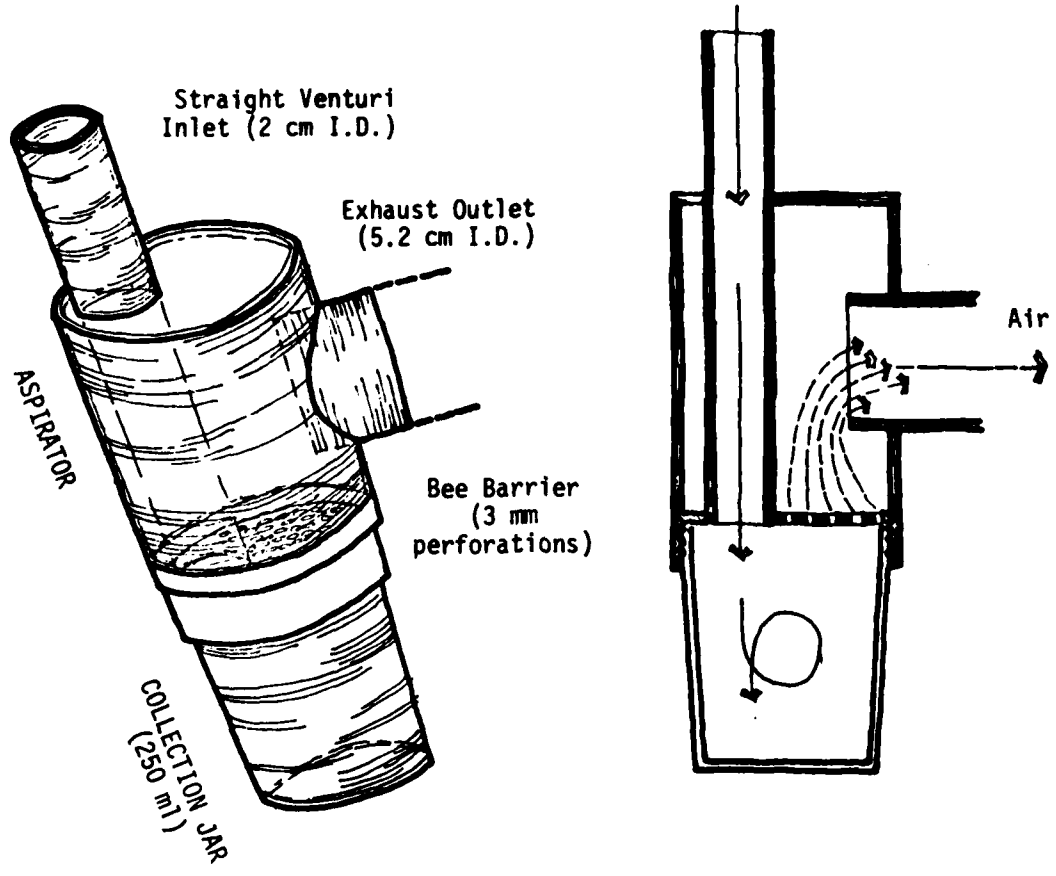

Figure 7-4. Acrylic Aspirator Used to Obtain Honeybees for Chemical Analyses

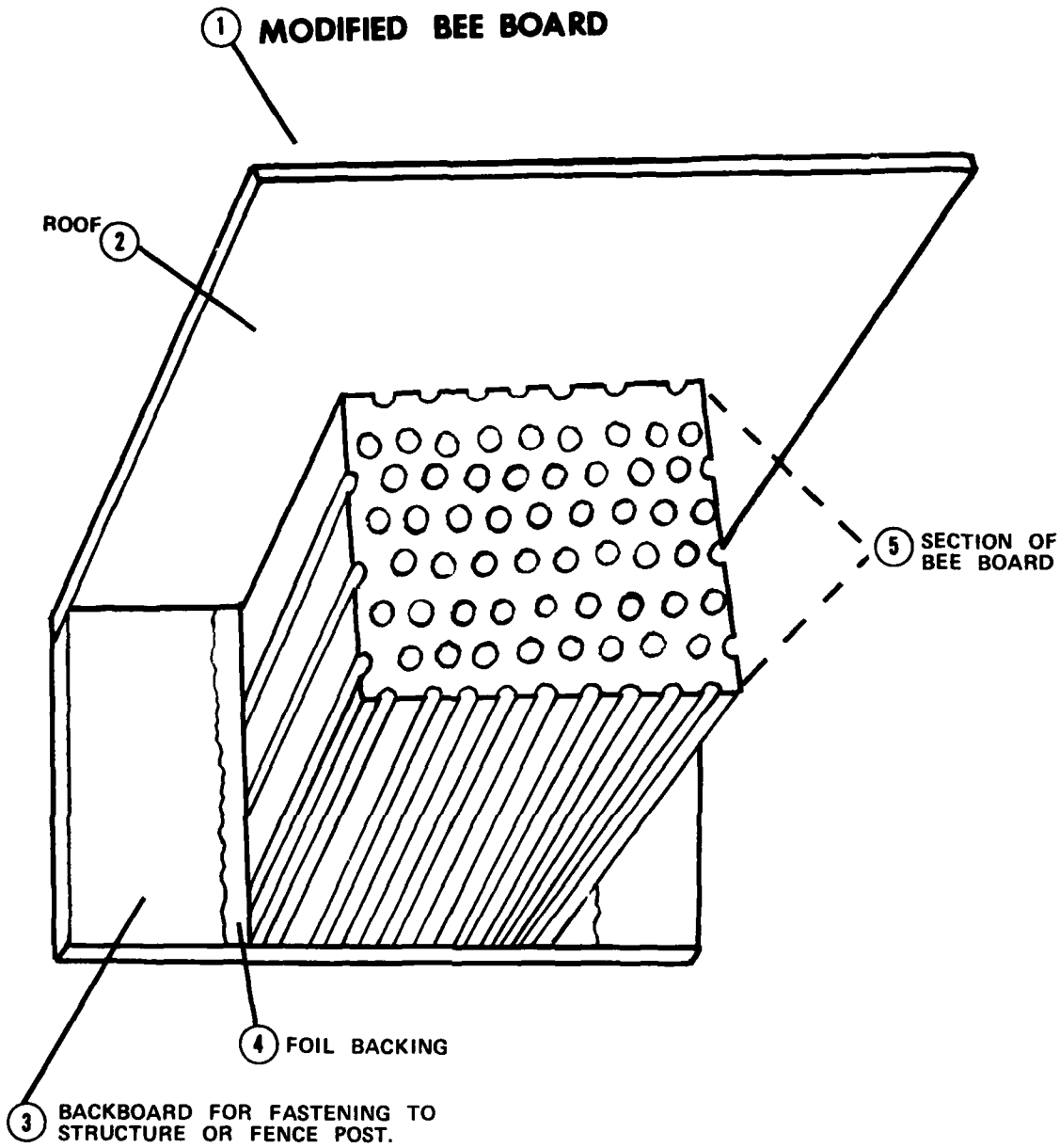

Figure 7-5. Modified Bee Board

7 centimeters (6 by 3 inches) and the top, 15 by 11.5 centimeters (6 by 4 inches). Both are fabricated from 6 millimeters(1/4 inch) exterior plywood. The piece of bee board contains nesting sites and consists of a block of wood measuring 7 by 7 by 8 centimeters (3 x 3 x 4 inches) with 51 holes 5 millimeters (3/16 inch) in diameter. Soda or drinking straws 5 millimeters by 7 centimeters (3/16 inch by 3 inches) are inserted into each hole. The soda straws (which must be of wax paper rather than plastic) assist in proper air exhange for the developing pupae. The rectangular plywood attached behind and over the bee board is for convenience in nailing the device to the surface of a building or post and for protection from the weather.

Another device (Figure 7-6) consists of elderberry or raspberry canes measuring roughly 45 centimeters (18 inches) in length and sharpened on the smaller end for insertion into the ground. Drill two 3 millimeter (1/8 inch) holes 15 centimeters (6 inches) apart on opposite sides of the cane and a 5 millimeter (3/16 inch) hole about 7 centimeters (3 inches) into the pith from the top. These holes facilitate occupancy by bees and wasps that nest above ground.

The observer should determine whether bee boards or canes are more effective. Bee boards or canes may be placed where they are protected by vegetation and where water is present or in other locations frequented by bees. The devices should be visited twice weekly and occupied cells counted and recorded. New devices should be put out when the old devices are approximately 3/4 full to accomodate new occupancies. Occupancy rates can be established by determining the number of new occupancies per unit time. These procedures apply mainly to leafcutter bees. Alkali bee nests consist of nest mounds (tumuli) in the ground, which, if located, may be observed for activity and counts of active burrows made.

Torchio[1] lists the following symptoms as indicative of poisoning in bees:

(a) An excessive number of dead or dying bees in front of the colony or dead bees on the floor of the hive during mild weather.

(b) An unusual number of dead colonies, particularly if they contain honey and pollen.

(c) A depleted population when the colony should be populous.

(d) Sudden cessation of food storage.

[1]U.S. Department of Agriculture, Agricultural Research Service, Washington, DC 20250. "Pesticides", Beekeeping in the United States, Agricultural Handbook Number 335, pp 97-101, P. F. Torchio, 1971.

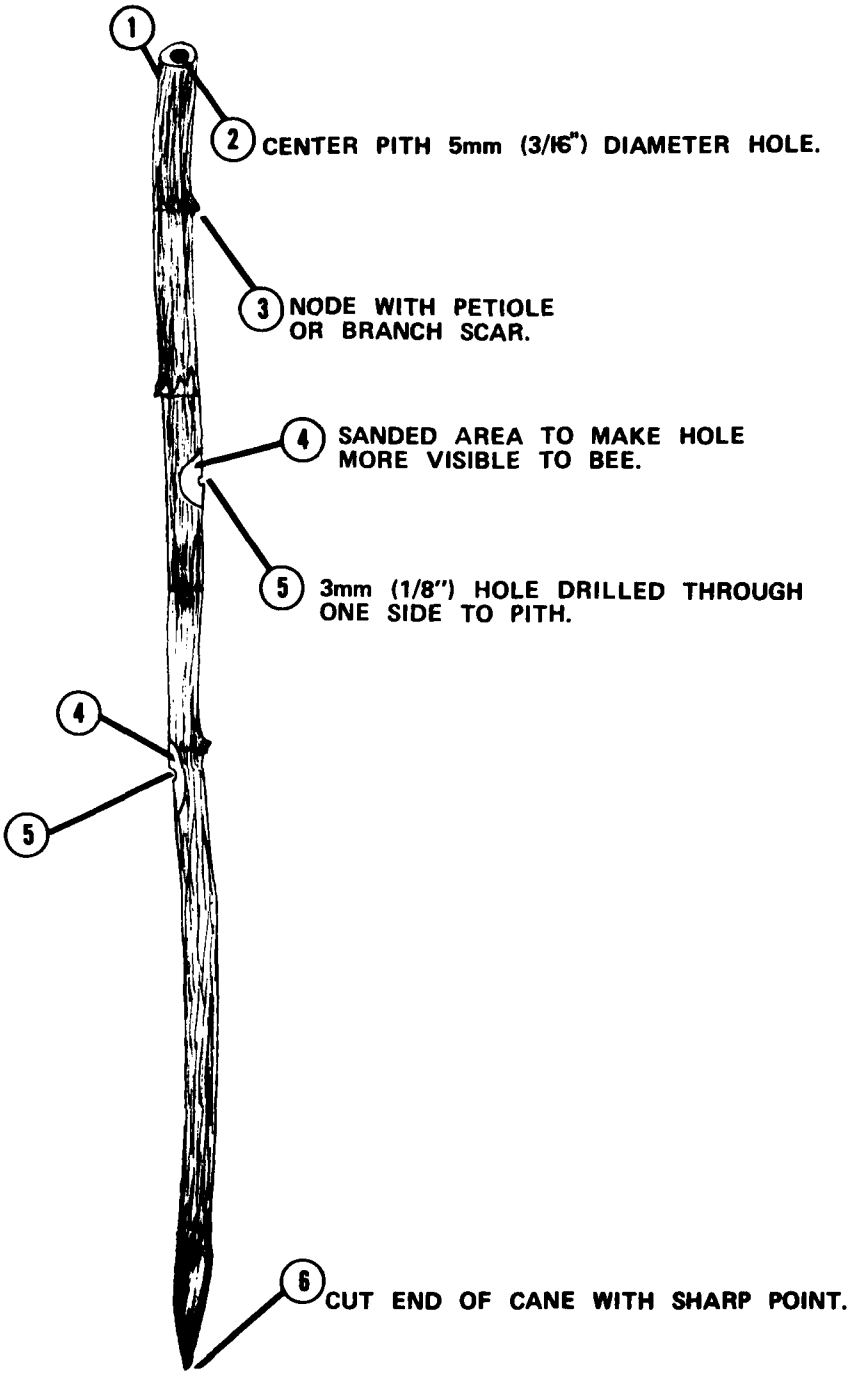

Figure 7-6. Nesting Cane

(e) Dead or deserted brood (offspring), with honey or pollen present.

(f) A severe break in the brood rearing cycle.

(g) Cessation of flower visitation (of significance where pollination is desired).

(h) Absence of the usual "hum" of workers in the air.

(i) Incoming nectar- or pollen-laden bees attacked at the colony by other bees.

(j) An unusual number of housecleaning bees emerging from the colony carrying dead bees. Much depends on colony size, season and activity.

(k) Paralyzed, stupefied or preening bees on weeds or other objects in the apiary.

(2) Record Keeping

You will receive training from the environmental scientists in species identification and may even receive a field key tailored to the species you will encounter. Refer to Appendix A.

Maintain a log of the survey (Appendix C)

Prepare maps of the survey sites (Appendix C)

Record samples on a standard label (Appendix C). Ignore the space for "Sex" and "Age". "Sample Type" will be "pooled individuals" "honey" or "pollen".

Record data from the counts on the field data sheet (Figure 7-7). The top row on the bee board is assigned the letter "A", the next row down is assigned the letter "B", etc., to the bottom row which is assigned the letter "F". The cells in each row are assigned consecutive numbers starting with 1 (one). Thus, occupied cells are indicated as: "A9", "C2", etc. Each day circle the occupied cells on the data sheet. Use each row for a separate cane.

Species_____ Collection Location_____
Collector_____ Colony Site_____
Starting Date_____ County_____
Ending Date_____ State_____

Dead Bee Counts

Day 1_____	Day 5_____	Day 9_____	Day 13_____	Day 17_____	Day 21_____
Day 2_____	Day 6_____	Day 10_____	Day 14_____	Day 18_____	Day 22_____
Day 3_____	Day 7_____	Day 11_____	Day 15_____	Day 19_____	Day 23_____
Day 4_____	Day 8_____	Day 12_____	Day 16_____	Day 20_____	Day 24_____

Bee Board or Cane Count

	Occupied Cells						New Cells
Day	Row A	Row B	Row C	Row D	Row E	Row F	
1	123456789	12345678	123456789	12345678	123456789	12345678	_____
2	123456789	12345678	123456789	12345678	123456789	12345678	_____
3	123456789	12345678	123456789	12345678	123456789	12345678	_____
4	123456789	12345678	123456789	12345678	123456789	12345678	_____
5	123456789	12345678	123456789	12345678	123456789	12345678	_____
6	123456789	12345678	123456789	12345678	123456789	12345678	_____
7	123456789	12345678	123456789	12345678	123456789	12345678	_____
8	123456789	12345678	123456789	12345678	123456789	12345678	_____
9	123456789	12345678	123456789	12345678	123456789	12345678	_____
10	123456789	12345678	123456789	12345678	123456789	12345678	_____
11	123456789	12345678	123456789	12345678	123456789	12345678	_____
12	123456789	12345678	123456789	12345678	123456789	12345678	_____
13	123456789	12345678	123456789	12345678	123456789	12345678	_____
14	123456789	12345678	123456789	12345678	123456789	12345678	_____
15	123456789	12345678	123456789	12345678	123456789	12345678	_____

Notes:

Figure 7-7. Field Data Sheet for Bee Surveys

(3) Refinement of Sample[1]

If analysis is for inorganic materials such as trace elements and radionuclides, store specimens in organic (plastic) containers. Zip-Lock[R] bags, Whirl Paks[R] (18 ounces) or their equal. For testing for organic materials, store specimens in glass containers washed with reagent grade acetone, hexane or ether and capped with aluminum foil and place these containers in an insulated ice chest with 10 to 12 kilograms (22 to 26 pounds) of dry ice.

When neutron activation is used for detecting and measuring trace elements, collection and storage equipment must not be washed with acetone. Rather use an acid (e.g. 1:1 nitric) followed by distilled or deionized water rinse.

(4) Shipping Refer to Appendix E.

(5) Equipment Checklist Refer to Appendix F.

Hives or mini-hives
4 penney nails
Hive entrance trap
Bee hat and veil
Smoker or mist sprayer
Sweep net
Dry ice in killing jar
Meter sticks
Hoe or square-end shovel
Aspirator
Predrilled nesting canes
Bee boards
Plastic or glass quart jars
6 mm wide, wood or porcelain spatula

[1]Specimens for chemical analysis can be sent to: Environmental Studies Laboratory (EVSL), Department of Botany, University of Montana, Missoula, MT 59801; or EPA Biological Investigations Laboratory, Beltsville, MD 20705.

CHAPTER 8 - RODENTS AND INSECTIVORES: INDICATORS OF PRESENT AND FUTURE AIRBORNE AND SOIL POLLUTION

CONTENTS

a. APPLICATION

Rodents and insectivores are good biological indicators for present and proposed polluting activities because: (1) the population has a high biotic potential thereby reflecting only recent environmental conditions; (2) they are sensitive to many pollutants[1]; (3) most do not hibernate and they are present in an area year round; (4) they have small home ranges; (5) they are usually abundant, and (6) they are easily sampled. Furthermore, rodents are primarily herbivorous and insectivora are omnivorous but mostly carnivorous. Therefore, different trophic levels can be sampled as the pollutant is biomagnified in the food chain. Other small mammals, e.g. carnivores, rabbits and bats, may posses most of the above characteristics; however, these groups are difficult to trap and handle, and therefore, have been excluded from consideration in this chapter. Rodents and insectivores can be studied in two ways: (1) species structure of the population and (2) tissue analysis for toxic substances.

b. SAMPLING TECHNIQUES

(1) General

All states require a permit to take mammals for scientific study. The procedures for obtaining permits are found in Appendix B.

[1]For example, laboratory mice are used to determine toxicity standards.

There are two principal types of traps: live traps and killer traps. The latter are used primarily for collecting museum specimen. and for destruction of small mammals as pests. Since in a PES, tissue from living specimens or recently living specimens is necessary for study, live traps only are considered here. Two types of live traps are suitable: box traps and pit traps.

There are several types of box traps, of these, sheet metal and open wire (Figure 8-1) are best suited for the purposes intended in this chapter.

Sheet metal traps are collapsible (folding) taking up less room during storage and transportation. They come in a size (23 x 8 x 8 centimeters - 9 x 3 x 3 inches) suitable for small rodents such as mice, chipmunks and small rats.

Open wire traps are also collapsible. They come in a size (50 x 16 x 16 centimeters - 19 x 6 x 6 inches) suitable for larger rodents such as squirrels, larger rats and prairie dogs.

Pit traps (Figure 8-2) capture a larger variety of animals than box traps. Pit traps are especially good for Insectivores. They consist of a partially buried solid metal or board drift fence with a one-gallon mayonnaise jar buried flush with the ground every 6 meters (20 feet).

In desert environments use rolled oats for bait. Place approximately a quarter handful into the rear of the sheet metal trap or drop the same amount through the top of the open wire trap onto the treadle. Some of the oats may fall off the treadle onto the ground but this should not pose a problem. In humid areas mix peanut butter with rolled oats until the oats have absorbed most of the oil of the peanut butter and the mixture can be rolled easily into balls approximetely 1 centimeter (1/2 inch) in diameter. Drop one such ball into the back of a sheet metal trap or place it firmly onto the treadle of an open wire trap by inserting your hand through the end of the trap. Pit traps do not require bait.

Set sheet metal traps by holding the trap in one hand and forcing the door open with the index finger of the other hand. Push the door with your finger all the way to the floor of the cage forcing the catch mechanism to the rear. Gently release the door until it is caught by the catch mechanism. Carefully put the trap down so as not to spring it. The trap can be made more sensitive by bending the catch mechanism slightly to the rear with the tip of the index finger or conversely, it can be made less sensitive by placing the index finger over the top of the catch mechanism and pulling it toward you slightly.

In humid areas sheet metal traps should be lined up, opened and hosed out to remove accumulated feces and rancid bait whenever necessary.

Set open wire traps by pushing the spring-loaded door-keeper down and raising the door. While holding the door against the top of the trap with one hand, pull the hook in the upper right corner of the open end over the wire of the door with the other hand. The hook is attached to the treadle which is raised into tripping position. Increase the sensitivity as necessary by rolling the hook back until it barely catches the door.

Set out the traps just before dusk. Check the traps early in the morning (particularly during summer when extended exposure to high temperatures can kill a trapped animal especially in the sheet metal traps). In winter each box trap must have a wad of cotton or similar material to provide protection from the cold. In deep grass place traps along rodent trails. In deserts and forests where there are few rodent trails place traps beneath some form of cover such as bushes or downed trees. It is wise to anticipate where morning shade will be to avoid baking the rodent as discussed above. Further assistance in learning likely spots to place traps will be provided by the environmental scientist. Unless a good spot is obvious, one place is as good as another to capture a rodent or other small mammal.

The most practical method of trapping rodents is to set trap lines. Trap lines will rarely need to be left in one place for longer than 1 week.

Identify the start of the trap line with a three-foot length of orange surveyor's tape tied to an easily spotted support such as a branch or the top of a clump of grass. Do the same with the end of the trap line. In dense vegetation it may be necessary to tag the location of each trap. Such marking facilitates theft, so consider the potential for a given area.

Depending on the density of available cover, sampling stations along the trap line should be at least 3 meters apart (three long steps), but not to exceed 10 meters. The trap line should contain as many traps as can be conveniently carried (20 to 40). As you walk along the trap line, drop one trap at each sampling station. On the way back (when the hands are free) unfold and set the traps.

After trapping is complete, remove the marking flags from bushes and trees.

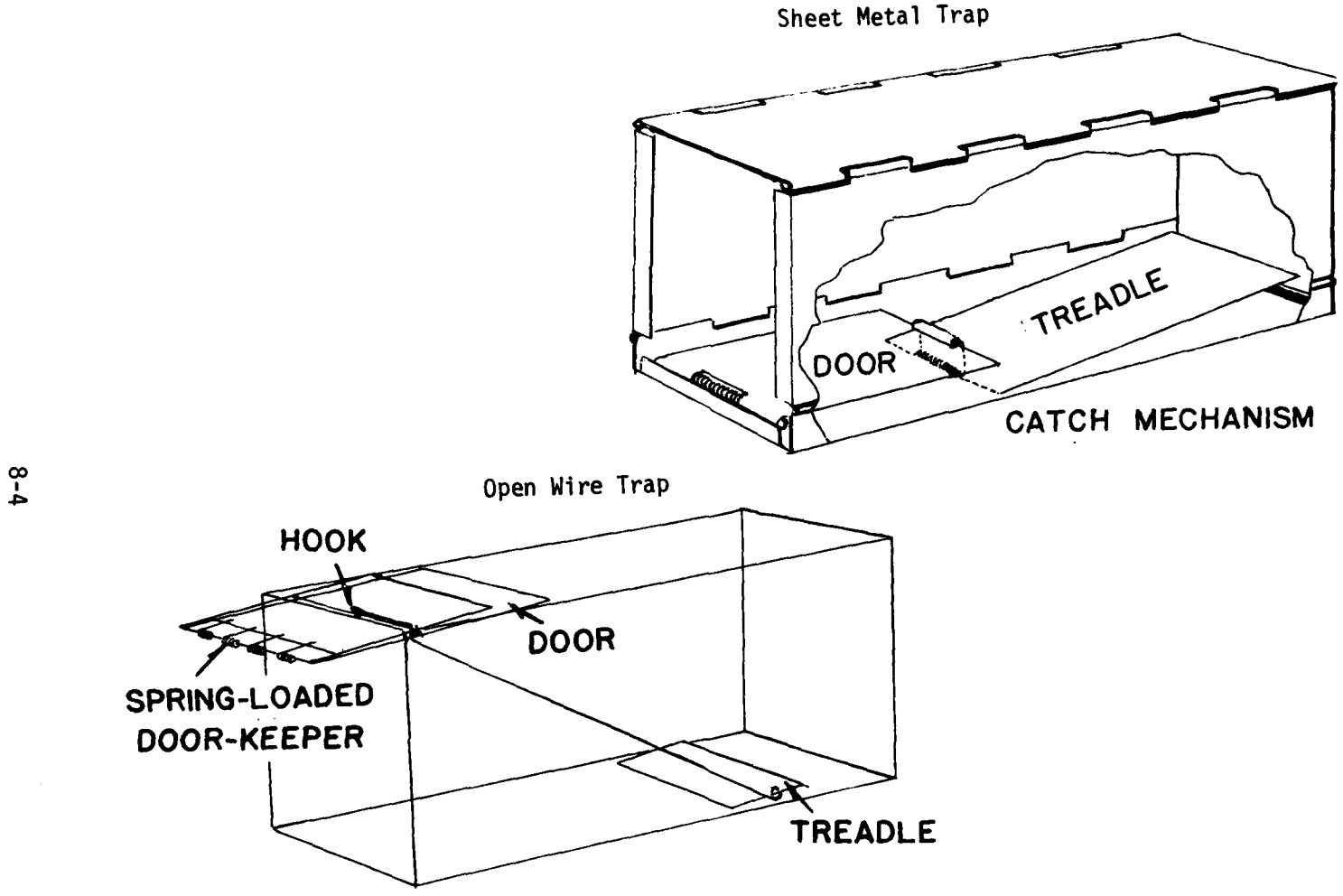

Figure 8-1. Sheet Metal and Open Wire Traps

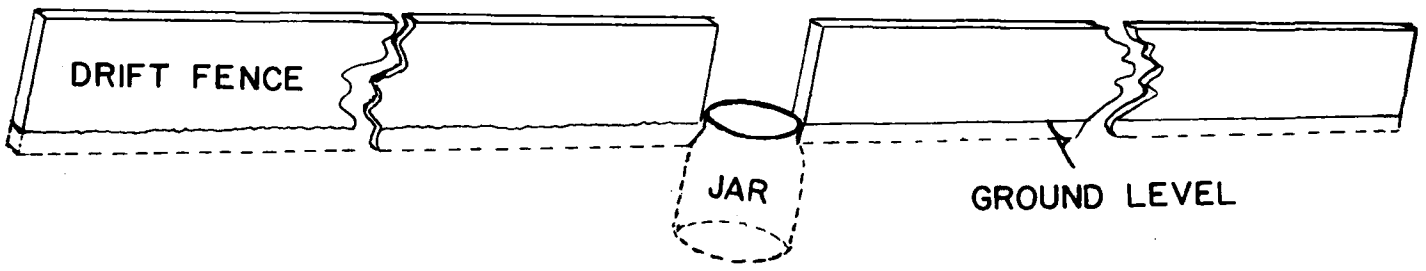

Figure 8-2. Drift Fence

Pit traps should be visted frequently (at least every 6 hours) as two or more Insectivora in the same jar will fight until only one remains alive. If dead specimens are satisfactory, the jars may be half filled with embalming fluid.

Handling trapped animals can be dangerous. All animals can carry diseases transmissible to man such as rabies, plague and tularemia, therefore, it may be necessary to be immunized against those endemic diseases commonly carried by the trapped species.

Small rodents will readily run from an opened box trap into a bag placed over the end. Since they prefer to escape by running uphill, tilt up the open end of the trap and the animal will soon be in the bag. This is preferable to shaking out the rodent together with bait, feces and sand. With an open wire trap, blow on the rodent to agitate him into running into the bag.

Insectivores and larger rodents may be removed from open wire traps or pit traps by placing a cloth bag over the hand and inserting the covered hand into the open wire or pit trap and grasping the animal gently. As the animal is removed, pull the bag over the eyes, this helps quiet the animal. The bag is then turned inside-out, the hand removed and the top of the bag tied.

(2) Record Keeping

As you lay out the trapline, locate it on the field map (Appendix C). If necessary, draw a larger scale map and include the location of each trap and prominent landmarks around each trap site. Attach these maps to your log. Also describe the habitat of the trapline in your log.

On subsequent visits to check the trapline, after bagging each trapped animal, weigh the bagged animal on the field balance. Identify the animal to species[1] and sex. If these data were not obtained while it was in the trap, remove the animal from the bag by carefully but firmly grasping the animal in one hand and peeling the bag away from him with the other until the identifying marks and sex organs are exposed. Make the required determinations, enter the data on the standard label and tape the left edge of the label to the log.

[1]Although you will receive training from the environmental scientists in species identification and may even receive a field key tailored to the species you will encounter, you also will need a general reference to the identification of species. Burt and Grossenheider is preferred for mammals. Burt, W. H. and R. P. Grossenheider, A Field Guide to the Mammals, Houghton Mifflin Company, Boston, MA, 1964. Also refer to Appendix A.

If the bagged animal is to be returned to your work area for identification or sampling, leave it in a shady and protected place until it can be processed according to the instructions in Part 4c(6), Volume 6, otherwise release the animal.

As the traps are checked, record in your log the number of traps sprung but empty. Also record the number alive, dead and released, by species, and if possible, the number escaped by species.

When you return to your workspace, attach the standard label to the log filling in all the remaining data. The ID number will include an identifier for the specific trapline and one for the individual trapped (e.g. A-3 for the third specimen captured in trapline A).

(3) Refinement of Sample Refer to Appendix D.

If the specimens are to be used for tissue analysis, follow the dissection procedures outlined in Part 4c(6), Volume 6.

(4) Shipping Refer to Appendix E.

(5) Equipment Checklist Refer to Appendix F.

Rodents	Insectivores
Container for box traps	8 in. by 20 ft. board
Pliers (for adjusting traps if necessary	Shovel
Rolled oats	Mayonnaise jars
Peanut butter	Embalming fluid
Bait container	Orange surveyor's tape
Cotton (winter time)	Knife
Orange surveyor's tape	Gloves
Knife	Cloth bags
Gloves	Field balance
Cloth bags (8 x 14 in.)	
Field balance	
Insecticide spray (for killing ectoparasites on rodents)[1]	

[1]Not to be used if ectoparasites are being collected for study.

APPENDIXES

APPENDIX A - IDENTIFICATION OF SPECIES

State or area animal and plant keys are used where available, for identifying species. Generally keys are available through local universities or the State Fish and Game Department. National guides to identification are found in Volume 2, Appendix B, Section V. Chapter VI, "Data Sources" in Study of Ecological Classification and Inventory Manual[1], provides a comprehensive listing of regional keys.

Even with the above aids, the team leader may determine that some taxa are too difficult to identify for all but trained taxonomists. These taxa will be prepared as directed and sent to the agency[2] designated by the team leader as per Appendix E.

[1]U.S. Department of the Navy, Naval Facilities Engineering Command, Alexandria, VA 22322. Study of Ecological Classification and Inventory Manual, M. M. Goodwin, Oct 1977.

[2]The Registry of Systematics Resources and Services (RSRS). Association of Systematic Collections, Museum of Natural History, University of Kansas, Lawrence, KA 66045, Phone: (913) 864-4867; FTS: 752-2312, is a data base that identifies names, addresses and phone numbers of specialists willing to provide a variety of services in the fields of plant and animal taxonomy. Ms. Rebecca A. Pyles is the point of contact.

APPENDIX B - PERMIT REQUIREMENTS

Federal and state permits are required to collect many types of live and dead animals and plants. The team leader will contact the Area Manager, Department of Interior (refer to Volume 6, Part 2) and the State Fish and Game Division or its equivalent to determine whether a collecting permit is required. If one is required and the team leader already has a valid collecting permit, all collections will be made under the provisions of his permit. If the team leader does not have the permit, he can obtain it through the Department of Interior, Area Manager, who will in turn go through his regional law enforcement division. The team leader may have to list the names of individual field workers collecting with him, and the numbers and names of the species to be collected. Accurate records must be kept since both federal and state agencies require an annual report listing the animals taken under each permit and their disposition.

Permits are required even if all collecting is confined to the military installation.

APPENDIX C - RECORD KEEPING

Log of the Survey

The log should be maintained in a bound notebook. Each page should be numbered consecutively. Each entry should begin with the date and time of the event described in the entry. It should end with the date and time the entry was made in the log. Always identify the location of the event, preferably by referring to a site number on a map (see below) in which case the map should be taped to the log book. Always identify those people accompanying you as witnesses to the event. Describe as much detail as possible - often seemingly insignificant observations may become important later on. Sign your name to the lower left corner of each page. All entries should be made with a fountain-type pen using waterproof ink. Erroneous entries should be lined-out with a single line. Each specimen sampled should be marked with a consecutive ID Number and that number should be entered into the log.

Map of the Survey

A map of the survey is essential and each member of the team should have a supply of maps so that he can locate each major place and event accurately, including sampling points and locations of observations. U.S. Geological Survey topographic map indexes, published for each state, Puerto Rico, the Virgin Islands, Guam and American Samoa, are available free from the U.S. Geological Survey, Washington, DC 20244 or the Federal Center, Denver, CO 80225. Appropriate quadrangles may be ordered at minimal cost from agencies identified on these indexes. Most facilities engineering directorates have all quadrangles for the installation. The maps may be purchased at sporting goods stores in some areas. Usually, the most desirable scale is the one showing the greatest detail of the area.

Standard Label

Fill out a standard label (Figure C-1) for each sample. Figure C-1 can be reproduced and the labels cut out for use. Explanation of entries is as follows:

"ID No." The identification number used to mark the sample.

"Species" The kind of mammal collected. (Appendix A)

"Sex" M, male or F, female. You will be trained to sex the animals collected.

"Age" J, juvenile; S, subadult or A, adult. You will be trained to age the animals collected.

"Collector" Your name (also name of other people present when the animal was collected).

"Coll date" The date the animal was collected.

"Coll Location-site" The site where the animal was collected. Refer to a place name found on your map. For example: "Orr Springs" "10 Km NNW Hatch Ranch" (Note: use metric system, Km-Kilometers and N,E,S,W, for North, East, South and West respectively).

Attach the label to the log (above). Beneath the label, enter other pertinent data such as weight, organs sampled and where shipped.

Photographs

Photographs, preferably both color and black-and-white, should be taken to record and substantiate conditions observed at the site of the survey. It is important to have some identifiable object in the picture to serve as a point of reference and as a scale to the area encompassed by the photo. The sun should be behind the photographer. Photographs taken between 1100 and 1400 give the best results. The use of a polaroid filter will reduce the glare of reflected light from the surface of water.

ID No_____ Coll Date_____

Species_____ Coll Location-site_____

Sex_____ Age_____ County_____

Collector_____ State_____

ID No_____ Coll Date_____

Species_____ Coll Location-site_____

Sex_____ Age_____ County_____

Collector_____ State_____

ID No_____ Coll Date_____

Species_____ Coll Location-site_____

Sex_____ Age_____ County_____

Collector_____ State_____

ID No_____ Coll Date_____

Species_____ Coll Location-site_____

Sex_____ Age_____ County_____

Collector_____ State_____

ID No_____ Coll Date_____

Species_____ Coll Location-site_____

Sex_____ Age_____ County_____

Collector_____ State_____

Figure C-1. Standard labels

APPENDIX D - PROCESSING OR REFINEMENT OF SAMPLE

Be sure the laboratory is willing to examine the samples. Let them know the approximate numbers and kinds of samples that will be sent, the types of analysis required, their date of arrival, the date the results will be needed and to whom the results should be sent. Also establish that your collecting and preservation techniques are compatible with their techniques.

APPENDIX E - SHIPPING

In general, the following will apply to material being shipped, however, check with the receiving laboratory to be certain that the procedure is compatible with their operation. Styrofoam coolers enclosed in boxes make the best shipping containers regardless of the method of preservation.

Shipping Method for Frozen Samples

Unless arrangements are made to receive samples on the weekends, samples should be sent by Tuesday. Early in the morning wrap all frozen specimens in insulating material, then place them in styrofoam coolers with at least 5 centimeters of dry ice between them and all sides of the box containers. Enclose two reproduced copies of each page (from the log) containing data of the specimens being sent. Securely close all openings in the box with masking tape. Label boxes in bold red letters: "frozen scientific specimens - do not store in warm areas", and "upon arrival at airport call _____". Put the number of the receiving laboratory in the blank space. Obtain a bill of lading from your transportation office and immediately take the boxes to the nearest air freight office and send them on the most direct flight to the airport nearest the receiving laboratory. Notify the laboratory by telephone that the specimens are on their way. Also provide the name of the airport, the airline, the flight number, the expected time of arrival and the bill of lading number.

Shipping Method for Non-Frozen Samples

The difference between this method and the previous one is that the space occupied by dry ice is filled with shock absorbing material and the label "Frozen scientific specimen, etc." is changed to read "Scientific specimens - store at room temperature".

APPENDIX F - GENERAL EQUIPMENT[1]

Vehicle (preferably 4-wheel drive)
Map of site (Appendix C)
Well-lighted work area with running water, distilled water, adequate bench space, telephone and 110 and 220V outlets
Log (Appendix C)
Standard labels (Appendix C)
Books or keys for the identification of specimens (Appendix A)
Binoculars or spotting scope
Fountain-type pen filled with indelible ink
Felt-tip, permanent ink pen
Collecting permits (Appendix B)
Photography equipment
  Camera
  Film
  Lenses (telephoto and wide angle)
  Filters
Clipboard
Shipping excelsior or shredded paper or dry ice
Shipping cartons (styrofoam coolers)
Masking tape
Bill of lading

[1]For Federal Supply Catalog number of some of these items, see Appendix I, U.S. Department of the Army, Methods of Preparing Pathologic Specimens for Storage and Shipment, TM 8-340, Sep 1963.

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